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THE KEEPING QUALITY, SOLUBILITY AND DENSITY OF POWDERED WHOLE MILK IN RELATION TO SOME VARIATIONS IN THE MANUFACTURING PROCESS. I. KEEPING QUALITY¹

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The volume of whole milk powder manufactured in the United States greatly increased during World War II. This product has not always been well accepted because under certain conditions it is subject to chemical or physical deterioration which may affect its palatability and reconstitutability in a relatively short period of time.

Many of the basic factors involved in the appearance of storage defects in whole milk powder are well known. However, further study is necessary to increase existing knowledge of the causative factors and thus to increase the shelf-life and consumer acceptance of whole milk powder.

REVIEW OF LITERATURE

A review of the literature indicates that high preheating temperatures of milk improve the keeping quality of powdered whole milk. The treatments reported as giving the best results varied considerably in the temperature-time ratio employed. The following heat treatments have been reported as beneficial in the production of whole milk powder of good keeping quality: 170 to 181° F. for 30 minutes (10, 11, 13, 24); 175° F. for 15 minutes (15); 190 to 195° F., without statement regarding time of exposure (20); 190° F. for 20 seconds, followed by a holding period of 2 to 3 minutes at a slightly lower temperature (17, 22); 220° F. for 10 seconds (15); and 250° F. for 1 second (24).

The reasons given for the effectiveness of the high preheat treatment by the above workers included: (a) the production of reducing compounds, namely sulphhydryls, and (b) more complete inactivation of enzymes. The

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production of sulphydryls through high preheat treatment is indicated by the work of other authors (6, 7, 16).

It has been shown that lecithin is easily oxidized, yielding a tallowy flavor (16, 23). In 1902 it was reported that temperatures of 203 to 230° F. destroyed as much as 26 per cent of the lecithin in milk (1), which may partially explain the improved keeping quality of whole milk powder made from high preheat-treated milk. Separate preheat treatment of cream and skim milk is associated with the fat and more particularly the lipids (20). Further evidence that lecithin may, in part, be responsible for the development of oxidized flavor has been produced by removing approximately one-half of the lecithin prior to powdering, which resulted in a better keeping powder even though the preheat treatment was only 160° F. for 30 minutes (2, 3).

Vacuum condensing of the milk improved the keeping quality of the powdered whole milk, apparently by removal of volatile catalysts (12). Condensing the milk to a high concentration was reported as desirable in the production of better-keeping powdered whole milk (24), and an increasing concentration of the milk—31, 38 and 45 per cent total solids—was shown to result in less retention of oxygen by the powder and an improved keeping quality (9).

All of the literature is in agreement that a low storage temperature improves the keeping quality of whole milk powder (4, 5, 8, 10, 14, 15, 17, 18, 19, 21). For instance, it was shown (4, 5) that, although little difference in the keeping quality of the whole milk powder resulted when the storage temperature range was 39.2 to 53.6° F., a very marked impairment of the keeping quality was evidenced in powders stored at 98.6° F. A study (14) with whole milk powders containing 1.54 per cent moisture showed an improvement in keeping quality as the storage temperature was progressively decreased from 86 to 38.4° F. A straight line relationship was indicated in the range of 77 to 50° F. Below 50° F. the improvement in keeping quality was more marked for each 9° F. decrease in temperature, and at 38.4° F. the rate of oxidative deterioration was one-half that found at 77° F. Lea *et al.* (17) considered storage for one day at 59° F. equivalent to 6 hours at 98.6° F. or 3 hours at 116.6° F.

EXPERIMENTAL PROCEDURE

The variables in the manufacturing procedure used in this study were limited to preheat treatment, precondensing and storage temperature. The four preheat-treatment levels studied were: 160 and 170° F. for 30 minutes and 170 and 180° F. for 10 minutes. For each run, morning milk in about 158-lb. quantities was obtained from the college herd and as nearly as possible from the same cows each day. Milk preheated at one of the levels indicated above was mixed thoroughly and divided into two equal parts.

One-half was then precondensed to approximately 20 per cent and the other to approximately 40 per cent total solids. The concentrated milk was homogenized at the condensing temperature and at 2,000 lb. pressure by means of a C. P. Multiflo homogenizer of 125-gallon capacity. The milk powder obtained from each lot of the concentrated milk was divided further into two lots and stored at 45 and 100° F.

The concentrated milk was atomized by air under 60 lb. of pressure and dried in an experimental spray drier. The spray nozzle was 1 mm. in diameter and was centered in the air outlet of 2 mm. diameter. The drying air, at about 255° F., flowed concurrently with that of the spray. The moisture-laden air was drawn from the drying chamber at approximately 160° F. through cloth dust collectors and then through a spray of cold water for dehydration by cooling.

The moisture content of the powder was determined by the vacuum oven method. Extremes in moisture content were 1.6 to 3.1 per cent, with most of the samples containing between 1.8 and 2.6 per cent moisture.

Immediately after drying the powder was manually mixed by means of a large spoon; 120-g. quantities were air-packed into no. 2 flat tins, hermetically sealed and stored at 45 and at 100° F. The powder was reconstituted on the basis of 1 part of powder to 7 parts of water and was scored by a panel of four judges 1 day after manufacture and at intervals of 1, 2, 4, 5, 6 and 10 months thereafter. For flavor scoring a previously unopened can was used. The following arbitrary scale of numerical values was used to rate the flavors: 1-2, bad; 3-4, poor; 5-6, fair; 7-8, good; 9-10, excellent. The "excellent" rating was awarded to samples free of any off flavors except for slight heated flavor. Whenever a heated flavor was detectable, the reconstituted milk was found not to be oxidized. The "good" rating was given to milk which was still quite acceptable in flavor, although it might have a very slight taint which could not be readily defined. The remainder of ratings were based on degrees of oxidized flavor.

RESULTS AND DISCUSSION

The changes in average flavor scores of the milk powder samples are shown in table 1. The effects of preheat treatments on the retention of palatability in air-packed milk powder as brought out here are in general agreement with the effects observed by other investigators. The powders made from milk preheated at 170° F. for 10 and 30 minutes and at 180° F. for 10 minutes deteriorated little in flavor during a 10-month period of storage at 45° F., but powders made from milk preheated at 160° F. for 30 minutes deteriorated in flavor rather rapidly during storage at 45° F. Preheat treatment of the milk at 170° F. for 10 minutes is not as effective as the more drastic preheat treatments in inducing good keeping quality in the powder, and treatment at 160° F. for 30 minutes is very ineffective. When

stored at 100° F., all of the powders deteriorated rapidly in flavor and became oxidized in flavor in 1 to 2 months.

Hetrick and Tracy (9) found that increasing preconcentration of the milk (31, 38 and 45 per cent total solids) resulted in powders of better

TABLE 1
Average flavor changes during storage

Powder from 20% concentrate			Powder from 40% concentrate		
Age	Av. flavor score after storage at		Age	Av. flavor score after storage at	
	45° F.	100° F.		45° F.	100° F.
Preheat treatment—160° F. for 30 min. ^a					
1 day	7.7	7.7	1 day	8.3	8.3
1 mo.	7.2	5.7	1 mo.	7.1	5.9
2 mo.	8.0	4.3	2 mo.	7.7	5.0
4 mo.	7.0	2.8	4 mo.	7.0	3.2
5 mo.	6.4	2.5	5 mo.	6.1	2.4
6 mo.	7.0	3.1	6 mo.	6.3	2.3
10 mo.	5.5	10 mo.	5.4
Preheat treatment—170° F. for 10 min. ^b					
1 day	8.0	8.0	1 day	7.9	7.9
1 mo.	8.1	6.2	1 mo.	8.4	7.0
2 mo.	8.3	4.9	2 mo.	7.9	5.4
4 mo.	8.0	3.3	4 mo.	7.7	3.9
5 mo.	7.9	3.6	5 mo.	7.8	4.6
6 mo.	7.7	2.5	6 mo.	7.5	3.6
10 mo.	7.2	10 mo.	7.3
Preheat treatment—170° F. for 30 min. ^a					
1 day	7.8	7.8	1 day	8.1	8.1
1 mo.	8.1	6.2	1 mo.	8.1	7.1
2 mo.	8.2	6.1	2 mo.	8.3	6.5
4 mo.	7.6	3.1	4 mo.	7.8	4.6
5 mo.	7.6	3.3	5 mo.	7.8	6.5
6 mo.	8.0	3.3	6 mo.	8.1	5.0
10 mo.	7.8	10 mo.	8.2
Preheat treatment—180° F. for 10 min. ^a					
1 day	8.3	8.3	1 day	8.6	8.6
1 mo.	8.3	6.6	1 mo.	8.3	7.3
2 mo.	8.1	5.9	2 mo.	8.3	6.9
4 mo.	7.8	3.6	4 mo.	7.8	4.9
5 mo.	7.9	3.7	5 mo.	8.1	4.7
6 mo.	8.0	3.2	6 mo.	8.1	4.4
10 mo.	7.8	10 mo.	8.2

^a Each score is the average of 6 samples.

^b Each score is the average of 4 samples.

keeping quality. From the results reported in the present paper, it appears that precondensing to 40 per cent total solids and preheat treating at 170° F. for 30 minutes or 180° F. for 10 minutes yields a powder with slightly better retention of palatability than does powder made from milk precon-

densed to the 20 per cent total solids level. The effect of concentration on the keeping quality was more marked when the powder was stored at 100° F. (table 1). However, at this temperature the improved keeping quality does not appear to be commercially significant.

CONCLUSIONS

1. Preheat treatment of the milk at 170° F. for 30 minutes or 180° F. for 10 minutes resulted in powders of good palatability even after storage, air-packed, for 10 months at 45° F. Preheat treatment at 170° for 10 minutes was slightly less effective for producing powders of good keeping quality. Air-packed powders made from milk preheated at 160° F. for 30 minutes deteriorated rapidly when stored at 45° F.

2. Milks preheated at 170° F. for 30 minutes or at 180° F. for 10 minutes and precondensed to approximately 40 per cent total solids resulted in powders which retained slightly better palatability during 10 months of storage at 45° F. than did powder made from the milk precondensed to approximately 20 per cent total solids.

3. All air-packed powders stored at 100° F. rapidly became oxidized in flavor. The powders made from milk preheat treated at 170° F. for either 10 or 30 minutes and at 180° F. for 10 minutes and precondensed to approximately 40 per cent total solids deteriorated in palatability slightly less rapidly than did the powders made from the 20 per cent preconcentrate.

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BENEFICIAL EFFECT AND ECONOMIC IMPORTANCE OF USING ALL COLOSTRUM PRODUCED IN CALF RAISING

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The high value of colostrum in the nutrition of the newborn calf has been well established (2), yet on most farms the colostrum not nursed by the newborn calf is wasted or not used for calf feeding. Practical methods of colostrum utilization must be devised and demonstrated to encourage its general use in raising herd replacements. This paper reports such a practical method.

Dann (4), in 1933, reported the vitamin A content of colostrum to be many times that of normal milk. Since then research workers have demonstrated repeatedly the importance of vitamin A in the nutrition of the newborn calf (5, 6, 8, 9, 10, 11, 13, 18). Colostrum also has been reported to be high in riboflavin (12, 15, 16), and a recent paper by Wiese *et al.* (17) indicates the newborn calf must have a dietary source of this vitamin.

In a recent paper on the physiological effects of extending the colostrum feeding period to seven days, the blood plasma level of vitamin A was reported to increase rapidly following the ingestion of colostrum, reaching a peak on the seventh day (14). The calves fed extra colostrum made more rapid weight gains and showed no signs of digestive disturbances during the colostrum feeding periods. The economic advantages of utilizing all colostrum in calf raising also were pointed out.

During the previous experiment, surplus colostrum was frozen and stored until used. On the average dairy farm this method for utilizing colostrum is not practical, since few farms are equipped to refrigerate and store any quantity of colostrum. Considerable inconvenience also is encountered in the feeding, since extra time and effort are required to prepare the colostrum for feeding.

The investigation presented in this report was undertaken to determine the effects of intermittent colostrum feeding for the duration of the milk feeding period. Observations were made of the effects on blood plasma vitamin A and carotene, weight gains and physical performance as indicated by condition of the animal. This experiment also demonstrates a practical method of colostrum feeding that could be followed on most dairy farms.

EXPERIMENTAL PROCEDURE

Calves born in The Ohio State University dairy herd between November 30, 1945, and December 1, 1946, were divided into two groups at birth.

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The five major dairy breeds and both sexes were represented within the groups. The calves were not permitted to nurse. During the first 3 days they were nipple-pail fed colostrum from their dams at a rate of 10 per cent of their body weights. After 3 days, the control group (Group II) was fed Holstein milk at the following rates: 10 per cent of body weight for the first 3 months, 8 per cent of body weight during the fourth month, 6 per cent of body weight during the fifth month, and 4 per cent of body weight during the sixth month. The group receiving extra colostrum (Group I) was fed according to the same schedule except that part or all of the Holstein milk was replaced by colostrum when available. The term colostrum as used in this experiment refers to the production of the first 3 days immediately following parturition. No effort was made to store any of the colostrum and it was used completely at each milking. A concentrate mixture, mixed hay of average quality and water were provided *ad libitum* to all calves up to 3 months of age. After 3 months of age the calves were fed the concentrate mixture twice daily and the amount increased until each calf was receiving approximately 4 lb. daily at 6 months of age.

All calves were weighed at birth, on the third day, and at weekly intervals during the experimental period. Calves were bled for vitamin A and carotene analyses at the time of weighing during the first 4 weeks. After that, the calves were bled at the end of each succeeding 4-week period. Blood samples were drawn according to a definite schedule without consideration of the amount of colostrum fed during the interval between bleedings. Blood plasma analyses for vitamin A and carotenoid pigments were determined by the method of Kimble (7). An Evelyn photoelectric colorimeter with appropriate filters was used for each determination.

EXPERIMENTAL RESULTS

A total of 76 calves, born in a 1-year period and made up of 19 Ayrshires, 17 Guernseys, 16 Jerseys, 15 Holsteins and 9 Brown Swiss, was used in this experiment. Twenty-two of the 36 calves starting the experiment in Group I were on experiment the full 24 weeks. Of the 40 calves in Group II at the beginning, 20 remained at the end of the 24-week period. The decline in numbers was primarily a result of selling bull calves and is without serious variation either in groups or breeds. Although a number of calves in both groups was afflicted with minor cases of scours, only 1 calf left the experiment as a result of calfhood diseases. This calf, a member of Group II, died of pneumonia.

During the experimental period 78 cows dropped calves and produced a total of 5,772 lb. of colostrum, or an average of 74.0 lb. per cow. Thirty per cent of the colostrum produced was used in feeding the calves during the 3-day period immediately following birth. The surplus, over 2 tons,

was used in feeding calves in Group I. Not all the colostrum used in this experiment was produced by the above cows, since colostrum feeding extended approximately 24 weeks beyond the time the last calf was allotted to the experiment. However, colostrum was produced during all seasons of the year and under both pasture and standard winter feeding conditions.

Table 1 presents the data on the amount of colostrum fed per calf during the first 3 days and for each succeeding 4-week period. The number of calves included in each average is tabulated. There is a wide individual variation in the amounts of colostrum received by the calves in Group I. One calf received as much as 445 lb., while another in the same group received as little as 50 lb. The variation in amount fed per calf resulted from the variation in calving dates and the sale of calves.

Data on the sex distribution, average weekly gains, and cumulative weight gains by groups are presented in table 2. It will be noted that the calves of Group I made more rapid and consistent weight gains during the

TABLE 1

Average amount of colostrum fed to each calf receiving extra colostrum for the first 3 days and by 4-week periods thereafter

	First 3 days ^a	4-week period					
		1	2	3	4	5	6
Lb. of colostrum fed ...	25.7	43.8	34.0	33.2	32.4	22.7	37.2
Av. no. of calves	36	35	32	26	25	19	17

^a Each control calf received 25.2 lb. of colostrum during the first 3 days.

first 4 weeks and maintained that advantage throughout the experiment, even though the number of male calves is greater in the Group II. Another important observation was that the calves in Group I exhibited a more thrifty appearance as indicated by their alertness, quality and gloss of hair coat, and greater vigor. Although a regular weighing schedule was followed, the variation in weekly gains was to be expected because feed and water were given *ad libitum*.

The average data on vitamin A and carotene changes in the blood plasma for each group and for breeds within the groups are presented in tables 3 and 4. The blood plasma levels for both carotene and vitamin A are extremely low at birth. This is in agreement with previously reported experiments (10, 14). The substantial increases in the blood plasma vitamin A and carotene noted on the third day are attributed directly to colostrum feeding. The early peak levels of vitamin A reached on the third day were consistent for both groups. These levels were not exceeded in either group until the sixteenth week. In Group II the early peak level of carotene in the blood coincided with the early peak level for vitamin A, but in Group I the early peak level for carotene was not reached before the

second week. One should note the higher blood plasma levels of vitamin A and carotene found for Group I from the third to the sixteenth week as compared with those for Group II.

At 4 weeks of age, when the lowest blood plasma vitamin A and carotene values are observed, a significant difference between groups was found. At this age the residual effects of colostrum feeding for the first 3 days are at a minimum, and the low roughage consumption does not greatly

TABLE 2
Sex distribution and average weight gains of calves in Groups I and II

Age of calf	Group I Extra colostrum				Group II No extra colostrum			
	No. & sex of animals		Av. weekly gain per calf	Av. cumu- lative gain of calves	No. & sex of animals		Av. weekly gain per calf	Av. cumu- lative gain of calves
	M	F			M	F		
(wk.)			(lb.)	(lb.)			(lb.)	(lb.)
1	16	20	3.8	3.8	22	18	2.8	2.8
2	16	20	4.7	8.5	22	18	4.0	6.8
3	16	20	5.7	14.2	22	18	5.3	12.1
4	16	20	5.7	19.9	22	18	5.9	18.0
5	13	20	7.8	27.7	20	18	7.9	25.9
6	12	20	8.2	35.9	17	18	8.4	34.3
7	12	20	8.8	44.7	16	18	8.0	42.3
8	10	20	9.1	53.8	13	18	9.7	52.0
9	8	20	13.0	66.8	12	18	11.5	63.5
10	8	20	11.3	78.1	11	18	10.9	74.4
11	8	20	12.3	90.4	11	18	12.3	86.7
12	8	20	13.3	103.7	10	18	13.0	99.7
13	8	20	13.3	116.0	9	18	13.8	113.5
14	8	20	12.5	128.5	8	18	11.8	125.3
15	8	20	14.8	143.3	8	18	12.8	138.1
16	7	20	13.5	156.8	6	18	13.1	151.2
17	7	20	15.7	172.5	5	18	15.0	166.2
18	7	19	15.9	188.4	5	18	13.9	180.1
19	7	19	16.0	204.4	5	18	13.1	193.2
20	5	18	13.0	217.4	5	17	13.8	207.0
21	5	18	17.9	235.3	4	17	13.0	221.0
22	5	18	15.0	250.3	3	17	18.8	239.8
23	4	18	12.3	262.6	3	17	10.9	250.7
24	4	18	15.0	277.6	3	17	14.0	264.7

influence blood plasma carotene and vitamin A values. This significantly higher level of vitamin A must be attributed to the colostrum feeding. The higher blood plasma carotene noted after the eighth week can be attributed to the hay consumption.

It is to be noted that a breed difference occurs at birth in the blood plasma levels of vitamin A and carotene. The blood plasma of the Guernsey calves in this study was significantly lower in vitamin A and higher in carotene at birth than that of the other breeds. No significant seasonal variation in the blood plasma vitamin A was found.

TABLE 3
Blood plasma vitamin A levels of calves used in the experiment
(Vitamin A expressed as γ per 100 ml.)

Group	Breed	Age in days						Age in weeks															
		1		3		1		2		3		4		8		12		16		20		24	
		No.	γ /ml.	No.	γ /ml.	No.	γ /ml.	No.	γ /ml.	No.	γ /ml.	No.	γ /ml.	No.	γ /ml.	No.	γ /ml.	No.	γ /ml.	No.	γ /ml.	No.	γ /ml.
I	Ayrshire	7	5.6	7	17.5	7	15.7	6	14.6	5	16.2	5	12.7	5	14.6	4	18.3	4	18.4	3	19.1	3	21.4
	Guernsey	7	3.2	7	16.9	8	14.7	8	11.7	8	10.2	8	9.9	7	10.2	7	14.0	7	18.8	6	19.8	6	27.9
	Holstein	8	5.0	8	14.2	8	13.5	8	14.0	8	12.6	8	13.1	8	13.5	7	14.8	7	15.3	6	16.1	5	18.5
	Jersey	6	5.0	6	15.2	6	13.6	6	14.6	6	14.3	6	10.4	4	12.2	5	17.6	5	19.8	5	20.7	5	24.5
	Brown Swiss	5	5.4	5	17.9	5	14.7	5	17.4	5	16.1	5	13.2	5	12.2	5	16.3	5	16.4	3	21.2	3	21.9
	Av.	33	4.8	33	16.2	34	14.5	33	14.7	32	13.4	32	11.8	29	11.8	28	15.6	28	17.3	23	19.1	22	23.2
II	Ayrshire	8	5.8	10	20.4	11	16.1	11	15.7	10	11.9	11	11.9	8	12.3	7	15.6	4	19.9	5	23.2	4	26.1
	Guernsey	9	3.3	9	14.7	9	13.1	9	12.3	9	10.1	9	9.0	7	9.7	7	12.9	6	14.6	5	23.6	5	23.7
	Holstein	6	4.9	6	14.9	7	12.1	7	11.2	7	11.8	7	12.7	7	12.6	5	13.5	5	18.1	5	17.4	4	20.4
	Jersey	8	5.1	7	13.1	9	11.4	9	12.6	9	12.3	9	9.7	8	10.1	7	13.9	5	20.0	4	25.2	4	23.3
	Brown Swiss	3	6.6	4	15.7	4	14.9	4	14.6	4	14.5	3	11.2	4	11.5	4	13.1	4	14.5	3	14.5	3	20.9
	Av.	34	4.9	36	16.1	40	13.5	40	13.3	39	11.8	39	10.8	34	11.2	30	13.9	24	17.3	22	21.2	20	23.1

TABLE 4
Blood plasma carotene levels of calves used in the experiment
(Carotene expressed as γ per 100 ml.)

Group	Breed	Age in days				Age in weeks																	
		1		3		1		2		3		4		8		12		16		20		24	
		No.	γ /ml.	No.	γ /ml.	No.	γ /ml.	No.	γ /ml.	No.	γ /ml.	No.	γ /ml.	No.	γ /ml.	No.	γ /ml.	No.	γ /ml.	No.	γ /ml.	No.	γ /ml.
I	Ayrshire	7	2.2	7	21.8	7	18.2	6	31.2	5	26.7	5	22.5	5	46.7	4	160.6	4	112.6	3	124.0	3	128.1
	Guernsey	8	5.0	7	65.1	8	72.2	8	73.2	8	61.0	8	60.0	7	104.2	7	162.4	7	253.4	6	235.6	6	267.5
	Holstein	8	3.5	8	25.4	8	32.2	8	40.4	8	30.1	8	29.1	8	32.2	7	55.0	7	76.0	6	101.2	5	141.5
	Jersey	5	3.7	6	32.1	6	33.9	6	41.6	6	40.5	6	25.2	4	41.4	5	122.8	5	212.8	5	200.5	5	171.5
	Brown Swiss	5	3.1	5	39.7	5	37.4	5	34.3	5	18.7	5	13.3	5	19.1	5	89.7	5	47.1	3	131.9	3	80.5
II	Av.	33	3.6	33	36.4	34	39.7	33	46.0	32	37.4	32	32.6	29	51.1	28	115.3	28	144.8	23	164.9	22	172.5
	Ayrshire	9	1.5	10	22.3	11	17.7	11	25.7	10	21.8	11	23.3	8	46.3	7	88.7	4	169.9	5	133.5	4	189.3
	Guernsey	9	4.8	9	75.2	9	66.9	9	72.4	9	60.6	9	65.7	7	109.1	7	152.8	6	213.8	5	267.8	5	337.6
	Holstein	6	2.3	6	25.6	7	28.4	7	29.9	7	22.6	7	18.8	7	39.5	5	54.4	5	86.8	5	84.7	4	145.7
	Jersey	8	4.0	7	38.1	9	34.7	9	26.1	9	26.9	9	17.7	8	70.8	7	193.1	4	194.9	4	318.0	4	267.2
II	Brown Swiss	3	2.3	4	44.4	4	34.0	4	21.1	4	15.4	3	12.0	4	17.8	4	45.4	4	60.8	3	128.2	3	114.2
	Av.	35	3.1	36	41.6	40	36.1	40	36.5	39	31.4	39	30.8	34	60.3	30	119.7	23	148.6	22	185.6	20	224.1

TABLE 5
The effect of the amount of extra colostrum fed on the blood plasma carotene
and vitamin A and on the cumulative weight gains

Amount of colostrum fed	Day		Week								Cumulated wt. gains	
	1	3	1	2	3	4	8	12	16	20		24
												(lb.)

Vitamin A γ /100 ml.

Carotene γ /100 ml.

Table 5 presents data showing the effects of feeding different amounts of colostrum on the blood plasma vitamin A, carotene, and cumulative weight gains of calves. It will be noted that the calves receiving more than 200 lb. of colostrum maintained higher levels of vitamin A in the blood plasma and made greater cumulative weight gains than those receiving less than 200 lb.

DISCUSSION

Considerable research effort has been devoted to the development of calf rations, especially those limiting whole milk consumption. The past world war, with huge demands for food, pressed this development. Research workers (3, 9) have developed limited milk feeding schedules using rations fortified with known essential vitamins. In these experiments colostrum was fed only during the first 3 days and no attempts were made to utilize further the surplus colostrum produced. Wise and LaMaster (19) suggested the use of colostrum and reconstituted skim milk in the feeding of young calves. However, they questioned the advisability of feeding colostrum to older calves.

Responses to colostrum feeding, reflected by the blood plasma levels of vitamin A and carotene, substantiate earlier reports (10, 14). In the experiment herein reported, calves fed surplus colostrum, whenever available, maintained higher blood plasma levels for vitamin A and carotene, made more rapid weight gains and exhibited a more healthy appearance than did the control calves. These calves showed more luster to the hair coat and were more active and alert, especially during the first 2 months. Feeding of surplus colostrum did not prevent cases of scours. Such cases occurred in both groups and resulted in marked drops in the blood plasma vitamin A but did not materially affect the results. As in the previous experiment (14), no cases of scours could be attributed directly to colostrum feeding, even though on many occasions rations were changed abruptly from complete milk rations to complete colostrum rations.

The economic importance of utilizing all colostrum in raising dairy calves is emphasized by this experiment. On the average, with five breeds and all ages of cows represented, the surplus colostrum produced amounted to 51.3 lb. per cow. In this experiment, with 78 cows represented, a total of 4,007 lb. of surplus colostrum was utilized in raising calves. If one-half as much surplus colostrum per cow from each of the 26 million dairy cows in the U.S.A. was utilized in calf raising, it would represent a saving of more than 650 million lb. of marketable milk. Allen (1) earlier recommended use of stored colostrum to replace marketable milk for raising dairy calves. The results of this experiment indicate that colostrum can be used to greatest advantage during the first month of life, when a calf must have milk in some form in its ration. During this period the calf needs special care and a well balanced ration if it is to survive and make thrifty

growth. After 4 to 6 weeks of age the feeding of extra colostrum has less marked effect because of greater consumption of hay and dry concentrate feeds. The consumption of these feeds; no doubt, also affects the rate of microbiological synthesis in the rumen.

SUMMARY

Seventy-six calves, born in The Ohio State University dairy herd, were divided into comparable groups at birth. For the first 3 days all calves received colostrum from their dams. After 3 days, the calves in both groups were fed and managed similarly, except that for calves in Group I, colostrum, whenever available, replaced part or all of the regular ration of Holstein milk. The amounts of either milk or colostrum fed were determined by the body weights of the calves.

Calves in Group I maintained higher levels of blood plasma vitamin A and carotene, made more rapid weight gains, especially during the first 6 weeks, and exhibited a superior physical appearance. Abrupt changes in the amounts of colostrum fed, which in some instances varied from no colostrum to all colostrum, did not create any special management problems. The calves did not scour from colostrum feeding.

Complete utilization of all colostrum for calf feeding is important from an economic standpoint. Only 30 per cent of the colostrum produced in The Ohio State University dairy herd during the calendar year was used in feeding calves during the first 3 days. The balance, which exceeded 4,000 lb., was used to replace an equal amount of marketable milk in the feeding of calves in Group I. The general practice of using all the colostrum produced in the raising of calves would result in a substantial saving of marketable milk.

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CAROTENE REQUIREMENTS FOR GUERNSEY AND JERSEY CALVES AS DETERMINED BY SPINAL FLUID PRESSURE

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Where calves of the various breeds are kept side by side, as in college herds, it has been noted that Guernsey and Jersey calves sometimes are more difficult to rear than Holstein or Ayrshire calves. The view has been expressed that this difference might be due to variations in carotene metabolism.

Boyer *et al.* (1) found that Guernsey calves required 57 γ of carotene per lb. of body weight to maintain a normal vitamin A level in the blood plasma, whereas Holstein calves required only 34 γ per lb. However, Nelson *et al.* (6) and Moore and Berry (4) did not note any difference in plasma vitamin A values from birth to 4 months of age between calves of the various dairy breeds, even when similar conditions of management were followed for all breeds. In these studies vitamin A as present in Holstein milk was fed to all the calves up to 2 months of age.

In order to gain further information on the question of whether there is a difference in the carotene requirements of the various breeds, the writers determined the carotene requirements of Guernsey and Jersey calves during the winter months, using cerebrospinal fluid pressure measurements as a criterion of adequacy.

The normal spinal fluid pressure varies between 75 to 120 mm. of water. Whenever the spinal fluid pressure exceeds 120 mm., it is considered abnormal and is taken as evidence that the calf is deficient in vitamin A. Previously published data (5) have shown that the spinal fluid pressure technique is quite precise and that it is possible to distinguish between differences in carotene intake of amounts as small as 2 γ per lb. of body weight. Where blood data are used, intakes must differ by as much as 10 to 15 γ per lb. before significant differences in blood values occur (5). In work on a considerable number of Holstein and Ayrshire calves, the authors never have observed an increase in spinal fluid pressure when 30 γ carotene per lb. were fed, whereas most calves on 28 γ show an elevated pressure. An increase above normal rather than the absolute value of the spinal fluid pressure is the deciding point in determining whether carotene intake is adequate.

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¹ The data for the Guernsey calves were collected while the senior author was located at the University of Maryland.

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EXPERIMENTAL PROCEDURE

Four Guernsey male calves and four Jersey male calves were used in this experiment to determine the minimum amount of carotene necessary to prevent an increase in spinal fluid pressure. The four Guernsey calves were fed carotene as present in dehydrated alfalfa-leaf meal at the rate of 28, 32, 34, and 36 γ per lb. of body weight, respectively, while the four Jersey calves were fed at the rate of 28, 30, 32, and 34 γ , respectively.

These levels of intake were maintained throughout the winter months, and with one exception all calves were placed on their respective levels of intake during the early fall or winter months. There are some differences in ages of the calves used on the experiment. Previous experience has not demonstrated any marked differences in requirements of calves varying from 4 to 14 months of age. Methods and procedures were the same as previously outlined (5).

RESULTS AND DISCUSSION

Table 1 shows the results for the Guernsey calves. The spinal fluid

TABLE 1

Results obtained with four Guernsey male calves

Date	Age	Weight	Plasma vitamin A	Plasma carotene	Spinal fluid pressure
	(days)	(lb.)	(γ /100 ml.)	(γ /100 ml.)	(mm. H ₂ O)
<i>No. 472 (Intake 28 γ carotene/lb.)</i>					
6-28-42	120	206	5.7	24
7-28-42	150	225	12.8	38	115
8-27-42	180	262	9.5	48	75
9-26-42	210	281	6.7	34	120
10-26-42	240	296	10.1	32
11-25-42	270	338	10.1	36	160
12-25-42	300	364	8.6	33	190
1-24-43	330	409	7.3	43	160
<i>No. 496 (Intake 32 γ carotene/lb.)</i>					
11-3-43	240	357	8.8	44
12-3-43	270	375	11.4	46	90
1-2-44	300	414	11.3	68	230
<i>No. 496 (Intake increased to 40 γ carotene/lb.)</i>					
2-1-44	330	456	14.1	71	160
3-2-44	360	487	12.8	67	90
4-1-44	390	542	90
<i>No. 89 (Intake 34 γ carotene/lb.)</i>					
11-15-44	120	9.1	19
12-15-44	150	182	13.8	39	90
1-14-45	180	209	10.9	54	100
2-13-45	210	236	10.9	67	100
<i>No. 90 (Intake 36 γ carotene/lb.)</i>					
12-15-44	120	181	15.0	29	90
1-14-45	150	206	12.5	49	100
2-13-45	180	241	10.4	45	90

pressure remained within normal limits throughout the experimental period for the two calves (nos. 89 and 90) that received 34 and 36 γ of carotene per lb. daily. At intakes of 28 and 32 γ (nos. 472 and 496) the spinal fluid pressure increased. When the carotene intake of no. 496 was increased to 40 γ per lb. after an increase in spinal fluid pressure had occurred, the spinal pressure values returned to normal in about 60 days. This effect may be partially seasonal, since unpublished data indicate that the cerebrospinal fluid pressure will start to decrease in March due to seasonal differences in carotene requirements. These data indicate that the minimum requirement for carotene by Guernsey calves is near 34 γ per lb. of body weight. The blood vitamin A values shown in this table also are of some interest. The vitamin A values for calf no. 496 during the period when his requirements were not being met (Nov. 3 to Jan. 2) were not noticeably lower than those for no. 89 or 90, and only in calf no. 472 were the vitamin A values below normal more or less consistently.

It will be noted that the spinal fluid pressure of calf no. 472 on the 28 γ level did not increase until he had been on experiment for about 4 months, whereas other calves showed increases in about 2 months. This calf was started during early summer and the delayed response is due to the fact that summer requirements (unpublished data) appear to be less than for the winter months.

Table 2 shows the results for the four Jersey calves. At levels of 32 and 34 γ of carotene per lb. of body weight, the spinal fluid pressure of calves no. 509 and 512 remained within normal limits (75 to 105 mm. H_2O). When the carotene intake of calves no. 2391 and 2392 was maintained at 28 and 30 γ , respectively, the spinal fluid pressure was above normal in both cases. Thirty-two micrograms of carotene per lb. of body weight therefore would appear to be the minimum requirement for Jersey calves, as compared with 34 γ per lb. for Guernsey calves. An examination of the plasma vitamin A values of these Jersey calves shows that these values were generally greater throughout the experimental period when carotene was fed at the highest level, but the differences in vitamin A values between calves no. 512 and 509, no. 509 and 2392, and no. 2392 and 2391 are so small that it is difficult to decide, on the basis of blood data alone, when the requirements actually were being met.

The requirement of 34 γ for Guernsey calves and 32 γ for Jersey calves is somewhat above the 30 γ level previously reported as being the minimum requirement for Holstein and Ayrshire calves. The differences in carotene requirement between the breeds therefore are not marked. In previous experiments with Holstein and Ayrshire calves there never has been an increase in spinal fluid pressure in calves fed as much as 30 γ or more of carotene per lb. of body weight. Yet in these experiments with Jersey and Guernsey calves, two receiving 30 and 32 γ showed elevated pressures. How-

ever, the 34 γ figure for Guernseys is much lower than that reported by Boyer *et al.* (1), whose data indicated a requirement of 57 γ of carotene per lb. of body weight for Guernsey calves and 34 for Holstein calves.

There are three possible explanations for this discrepancy. Boyer *et al.* (1) utilized blood plasma values as a criterion of adequacy. A study of the numerous data collected from this laboratory has shown that there

TABLE 2
Results obtained with four Jersey male calves

Date	Age	Weight	Plasma vitamin A	Plasma carotene	Spinal fluid pressure
	(days)	(lb.)	(γ /100 ml.)	(γ /100 ml.)	(mm. H_2O)
<i>No. 2391 (Intake 28 γ carotene/lb.)</i>					
8-29-46	200	285	5.7	39
9-28-46	230	339	5.5	56	95
10-28-46	260	364	6.5	55	170
11-27-46	290	415	7.8	58	165
12-27-46	320	448	7.9	89	185
1-26-47	350	465	9.7	102	175
<i>No. 2392 (Intake 30 γ carotene/lb.)</i>					
8-31-46	200	231	4.5	68
9-30-46	230	247	6.8	94	135
10-30-46	260	294	7.8	106	160
11-29-46	290	321	9.0	99	195
12-29-46	320	342	9.1	106	165
1-28-47	350	396	8.6	110	210
<i>No. 509 (Intake 32 γ carotene/lb.)</i>					
8-27-46	190	247	6.4	83
9-26-46	220	275	6.2	107	75
10-26-46	250	321	8.1	134	80
11-25-46	280	402	9.2	138	75
12-25-46	310	431	11.9	175	75
1-24-47	340	440	10.0	146	80
<i>No. 512 (Intake 34 γ carotene/lb.)</i>					
8-31-46	170	173	7.2	42
9-30-46	200	213	8.0	51	100
10-30-46	230	237	8.8	77	90
11-29-46	260	300	10.6	62
12-29-46	290	327	10.8	110	105
1-28-46	320	378	12.7	103	85

must be a spread in intake of at least 10 to 15 γ of carotene per lb. of body weight before a good correlation with blood data can be noted. The blood data in tables 1 and 2 further emphasize this point. Calves on the same intake show considerable individual variation in plasma vitamin A values. There also may be differences in requirements between various strains or families of Guernsey cattle. If inheritance plays some part in causing higher carotene requirements for Guernsey than for Holstein

calves, it is probable that there would be differences in families or strains of Guernseys. This point should be investigated. Another possible cause for the difference between the results from this laboratory and those from Wisconsin might be the difference in some environmental factor. Unpublished data show a higher requirement for the winter months than for the summer months.

The vitamin A content of the blood plasma of the Guernsey and Jersey calves used in this experiment is of the same order as that of the Holstein and Ayrshire calves that were fed at similar levels of carotene intake in previous experiments (5). This would indicate further that the carotene requirement of the Guernsey and Jersey calves used in this experiment was not greatly different from that of the Holstein and Ayrshire calves. Even though the difference is not great, it might account for some of the difficulty that sometimes is encountered in raising Guernsey calves, particularly if the hay quality is very poor or hay consumption is not adequate.

Requirements reported in this experiment are about double those reported by Moore (3) and Guilbert *et al.* (2), where night blindness was used as a criterion. The latter authors term their requirements "physiological minimum". In the light of the present and previous data (5), such an interpretation no longer is tenable. Furthermore, minimum requirements should be based on the least determinable change produced in the animal by a deficiency of the nutrient under study. The carotene requirements for calves as determined by cerebrospinal fluid pressure would appear to meet this definition more closely than night blindness, since changes in spinal fluid pressure occur before night blindness can be detected. The requirements as determined by the night blindness technique therefore cannot be considered adequate.

SUMMARY

1. Guernsey calves under our experimental conditions required an intake of 34 γ of carotene per pound of body weight during the winter months to maintain a normal spinal fluid pressure. On the same basis, Jersey calves required an intake of 32 γ . If inheritance is found to affect carotene requirements, it is possible that these values might need to be modified even for strains within the breed.

2. This requirement is slightly higher than for Holstein and Ayrshire calves, which, under similar conditions, require 30 γ of carotene per lb. of body weight.

3. These results appear to indicate that somewhat more attention should be given to the quality of hay used in feeding Guernsey and Jersey calves than in feeding Holstein or Ayrshire calves.

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MANUFACTURE OF POWDERED CREAM FOR WHIPPING BY AERATION¹

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A number of contributions were made during the war to the knowledge of methods for the successful manufacture of powdered whole milk and ice cream mix. Because of the success of industry with these two items, it is logical to consider the application of dehydration to certain other types of dairy products, such as cream. In 1922 Babcock (2) demonstrated that reconstituted powdered cream containing as much as 40 per cent fat failed to whip. He concluded that such a product may be considered as useless for whipping purposes.

In 1937 Getz *et al.* (3) reported on a method for whipping cream by aeration. The cream is charged with nitrous oxide at a pressure of about 175 lb. in a specially constructed steel container. The cream under pressure is released to the atmosphere through a Schrader valve,² resulting in a greatly expanded volume. While this method involves gasification of the serum portion of the cream, air incorporation in ordinary cream whipping is dependent upon a partial clumping of the fat. Because of this fundamental difference, it was thought that the process of drying cream to be whipped by aeration would have little or no effect upon either the gas dispersion or the stiffness of the whip. This study was made to test this hypothesis.

EXPERIMENTAL PROCEDURE

The cream-mix was made from fresh sweet cream, condensed skim milk, skim milk, sugar, stabilizer and flavoring. It then was pasteurized at temperatures not over 160° F. for 30 minutes and spray dried. Attempts were made to keep the iron and copper content at a minimum. The importance of each of the following factors was studied: (a) composition of the cream-mix; (b) nozzle size; (c) spray pressure; and (d) relation of inert gas and certain antioxidants to keeping quality.

Some of the experiments reported herein were conducted during World War II with a product containing 19 per cent butterfat on a reconstituted basis. Unless otherwise stated, the remainder of the experiments have been conducted with mix containing approximately 30 per cent butterfat on a

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¹ Process and container patented by Aeration Processes, Inc., Columbus, Ohio (U. S. Patent nos. 2,294,172 and 2,281,604).

² The Schrader valve originally employed has been superseded by an entirely new valve of original design (Model F2). This newly constructed valve meets the specifications of the U. S. Public Health Service.

reconstituted basis. Sucrose was used as the sweetening agent and was added before drying. Pure vanilla concentrate was used for flavoring the cream. The cream-mix was not condensed before drying, as the total solids content made this procedure unnecessary. All the cream-mixes were dried on an experimental spray drier using a no. 72 nozzle with a no. 20 core unless otherwise stated.

In the keeping quality studies, the powdered cream-mix was uniformly mixed and packed in no. 1 tin cans, the same weight of dried cream-mix being put into each can. After sealing, the cans were gas packed where necessary for the experiment.

For whipping purposes the dried cream-mix was reconstituted in the proper ratio with cold tap water and held for 24 hours or more at 40° F. Eight ounces of cream-mix were placed in Instantwhip³ containers, which then were charged with nitrous oxide gas and chilled before dispensing. The dispensed product usually was examined for drainage, overrun, flavor, body and appearance. Drainage was measured by dispensing an Instant-whip container into a 6-inch funnel, fitted with a screen at the apex, and set in a graduated cylinder (fig. 1). The volume (ml.) of drainage secured in 30 minutes at room temperature was recorded as the measurement of the amount of drainage.

On the powdered samples held in storage, changes in oxygen concentration in the headspace gas and changes in palatability were obtained at various intervals. The oxygen values were secured by the Manometric procedure of Van Slyke and Sendroy (12) and the flavor scores by two or more judges using the ice cream score card based on a flavor score of 45 out of a possible 50 points.

Moisture was determined by the toluol distillation method (1), butterfat of the powdered cream-mix by the Mojonnier method (8), copper by the procedure of Hetrick and Tracy (5), and iron by the method of Pyenson and Tracy (9).

RESULTS

Effect of variations of butterfat on whipping properties and appearance of reconstituted powdered cream-mix. Cream-mixes containing 26, 30, 32 and 34 per cent butterfat were prepared (batches no. 1, 2, 3 and 4, respectively). The milk solids-not-fat were standardized to 7.5 per cent. Other ingredients added were 5 per cent sugar, 0.3 per cent emulsifying agent and 0.1 per cent vanilla. After drying, a portion of each of the four lots was reconstituted with the proper amount of water, aged for 24 hours, and then placed in the containers, gassed and chilled before dispensing. The samples were shaken uniformly and dispensed simultaneously. There was only 1.5 ml. drainage from the 26 per cent product, 0.5 ml. drainage

³ "Instantwhip" is the trade name for the product as distributed by Aeration Processes, Inc., Columbus, Ohio.

from the 30 and 32 per cent products and no drainage from the 34 per cent product. The flavors of all the samples were identical. As the fat content was increased from 26 to 34 per cent, an improvement in the body and texture resulted and the product became drier in appearance. Since only a slight advantage resulted from using 32 or 34 per cent fat cream-mix as compared with the 30 per cent fat cream-mix, it was decided that future trials with heavy cream-mix would be limited to mixes containing 30 per cent butterfat.

Another factor that influenced the decision to standardize on 30 per cent butterfat was the heavy viscosity obtained with the higher-testing samples. Reconstituted cream-mix made from dried cream has a greater viscosity than the original cream-mix before drying. Too great a viscosity interferes with filling and the containers must be shaken longer to dissolve the nitrous oxide gas in the cream.

Effect of variations in the milk solids-not-fat on whipping properties and appearance of reconstituted powdered cream-mix. The mixes for this study were standardized to contain 30 per cent butterfat, 5 per cent sugar, 0.3 per cent emulsifying agent and 0.1 per cent vanilla. The variations in the milk solids-not-fat were 6, 7, 8 and 9 per cent (batches no. 5, 6, 7 and 8, respectively). Concentrated skim milk was used to increase the percentage of non-fat milk solids.

On reconstitution, the sample containing the 9 per cent milk solids-not-fat had the greatest viscosity, while the sample with the normal milk solids-not-fat (6 per cent) had the least viscosity. Increasing the milk solids-not-fat produced a heavier body in the whipped cream-mix. The flavor and standing-up qualities of the whipped cream-mix also were improved by the additional milk solids-not-fat.

Drainage from the whipped cream-mix was reduced by increasing the milk solids-not-fat content of the cream-mix. However, 9 per cent milk solids-not-fat in the 30 per cent butterfat cream-mix reduced overrun and produced a moist body. Since too great a viscosity produces mechanical difficulties in filling the containers and since too moist an appearance is produced by more than approximately 8 per cent milk solids-not-fat, it was deemed advisable to limit the milk solids-not-fat content to approximately 8 per cent in 30 per cent butterfat cream-mix.

Comparison of whipped reconstituted powdered cream-mix made with and without added emulsifying agent. These comparisons were made using several emulsifiers. Typical results of a representative emulsifying agent are presented. Cream-mixes containing 29.5 per cent butterfat, 7.5 per cent milk solids-not-fat, and 5 per cent sugar were prepared without and with 0.2 per cent glycerol monostearate. These mixes (batches 9 and 10) were dried and reconstituted in the usual manner. There was no drainage at room temperature in either sample in 0.5 hour. The flavors of both

samples were identical. The sample containing the emulsifying agent whipped to a higher overrun at any given pressure and the whipped cream had a drier appearance, a better body and a finer texture than the control sample containing no emulsifier. Commercial products containing emulsifying agents such as sorbitan monostearate also were found to be satisfactory. Gelatin and sodium alginate stabilizers alone were not satisfactory for powdered cream-mix for whipping purposes. They produced a moist appearance in the whipped cream.

Effect of nozzle size on whipping properties of reconstituted cream-mixes. A mix containing 29 per cent butterfat, 7 per cent milk solids-not-fat, 5 per cent sugar and 0.3 per cent emulsifying agent was used. The nozzles used were nos. 79, 72 and 65, representing openings of 0.0145, 0.025 and 0.035 inch, respectively (batches 11, 12 and 13).

There were no significant differences in whipping properties, body and texture or appearance of whipped reconstituted cream-mixes sprayed with the different nozzles. The capacity of the drier was lowered by using nozzles with smaller orifices. The packing density was increased by using nozzles with relatively large orifices.

Relation of spray pressure to the whipping properties of reconstituted powdered cream-mix. Homogenization is detrimental to the body, texture, appearance and drainage of cream whipped by aeration. The lower the fat content of the cream, the more detrimental is the effect of homogenization (10).

To determine whether the spraying process has any detrimental effect on the whipping properties of reconstituted powdered cream-mix, a lot of cream-mix (19 per cent butterfat, 9 per cent milk solids-not-fat, 6 per cent sugar, 0.15 per cent gelatin, and 0.2 per cent emulsifying agent) was divided into three portions and sprayed at: (a) 200 lb. pressure, (b) 1,500 lb. pressure, and (c) 2,800 lb. pressure (batches 14, 15 and 16).

The amount of spray pressure used had no significant effect on the overrun obtained on powdered cream-mix whipped by aeration (table 1). The drainage, however, was nearly doubled as the pressure was increased from 200 to 2,800 lb. As the spray pressure was increased, the whipped cream-mix became more moist in appearance and contained larger gas cells. Similar results were obtained with 30 per cent reconstituted powdered cream-mix, except that the detrimental effect of the higher spray pressures was not as pronounced as with 19 per cent cream-mix.

Relation of inert gas and certain antioxidants to keeping quality. In the commercial manufacture of powdered whole milk, replacing air in the package with nitrogen and/or carbon dioxide has been found to decrease the intensity of the oxidized flavor over a period of time and, in some cases, delay onset of oxidized flavor development. In preliminary trials it was shown that dried cream-mix packed in air could be held only a few weeks

at room temperature before a stale or oxidized flavor developed. To have commercial value, it is necessary that dried cream-mix keep for longer periods of time. That the oxidized flavor can be delayed by the addition of certain antioxidants to milk before drying has been demonstrated by Hollender and Tracy (6), Tracy *et al.* (11), Jack and Henderson (7), Waite (13), and Hetrick and Tracy (5).

To determine whether the use of antioxidants would prolong the keeping quality of dried cream-mix, six 50-lb. lots of cream-mix (29.5 per cent butterfat, 8 per cent milk solids-not-fat, 5 per cent sugar and 0.2 per cent glycerol monostearate) were dried, containing the following levels of antioxidants. The indicated percentages represent the amounts used as calculated on the basis of the weight of the fat: Batch no. 17, control, no added antioxidants; 18, Viobin antioxidant, 0.1 per cent; 19, nordihydroguaiaretic acid (NDGA), 0.03 per cent; 20, gallic acid, 0.1 per cent; 21, sodium

TABLE 1

Effect of spray pressure on whipping properties of reconstituted powdered cream-mix

Batch no.	Spray pressure	Overrun	Drainage	Firmness	Gas cell structure	Dryness
	(lb.)	(%)	(ml.)			
14	200	500	22	Good	Small uniform gas cells	Dry
15	1,500	490	39	Poor	Large irregular gas cells	Moist
16	2,800	510	43	Very poor	Large irregular gas cells	Moist

arabo ascorbate, 0.1 per cent; and 22, natural mixed tocopherols, 0.1 per cent.

All of the antioxidants were added directly to the cream-mix at the preheater just before spray drying, except the NDGA, which was dissolved in 15 mm. of butter oil before adding to the preheater. Both air- and nitrogen-packed samples were prepared. The samples were stored at room temperature. Data taken during 6 months of storage are given in table 2.

Oxygen concentration in the headspace gas gradually was lowered and the dried cream became less palatable as the storage period advanced. A stale flavor usually preceded the oxidized flavor in both air and nitrogen packed samples. After 180 days the nitrogen-packed control sample, although slightly oxidized, was still palatable, while the air-packed control sample had a pronounced oxidized flavor.

At the end of 6 months of storage at room temperature, all air-packed batches containing antioxidants were oxidized except batch no. 22, which had become intensely metallic⁴ after 5 weeks of storage. Through the 121-day storage period, the air-packed batches containing antioxidants scored

⁴ Not to be confused with the typical oxidized or tallowy flavor.

consistently higher than the control that contained no antioxidant. The addition of antioxidants to powdered cream-mix, especially when nitrogen packed, increased the keeping quality of the powder. The most effective antioxidants were NDGA, gallic acid and Viobin. The sodium arabo ascorbate delayed onset of the oxidized flavor but produced a cooked or "nutty" flavor in the cream-mix, which persisted throughout the storage period. Samples containing mixed tocopherols developed a very metallic off-flavor after 5 weeks of storage.

TABLE 2

Changes in oxygen concentration in head space gas and palatability of air-packed and gas-packed dried cream containing antioxidants

Batch no.	Antioxidant		Days storage at room temperature					
			7	14	35	58	121	180
17	Control	% oxygen	20.46	20.62	20.14	19.78	18.79
		Flavor	45	41	39	38.5 ^b	37.5
17N ^a	Control	% oxygen	0.85	1.06	0.84	0.68	0.62	0.28
		Flavor	45	42	41 ^b	40.5	40
18	Viobin	% oxygen	20.11	20.56	20.56	19.77	19.22
		Flavor	45	43	41	40	39.5 ^b
18N	Viobin	% oxygen	1.03	0.76	1.11	0.47	0.52	0.51
		Flavor	45	43.5	42	42	42
19	NDGA	% oxygen	20.0	20.12	20.44	20.11	19.84
		Flavor	45	43.5	43	43	41 ^b
19N	NDGA	% oxygen	1.08	1.02	0.95	1.03	0.19	0.33
		Flavor	45	44	43	43	42.5
20	Gallic acid	% oxygen	20.04	20.35	20.53	20.07	18.11
		Flavor	45	44	43	43	40 ^b
20N	Gallic acid	% oxygen	0.92	0.50	0.51	0.54	0.45	0.21
		Flavor	45	44.5	43.5	43.5	42
21	Sodium arabo ascorbate	% oxygen	20.35	20.3	19.91	19.64	18.26
		Flavor	42.5	42.5	42	41	40 ^b
21N	Sodium arabo ascorbate	% oxygen	1.06	0.71	0.76	0.65	0.56	0.00
		Flavor	42.5	42.5	42.5	42.5	42
22	Natural mixed tocopherols	% oxygen	20.39	20.35	20.09	16.29	18.32
		Flavor	41	35 ^c	33 ^c	30 ^c	25 ^c
22N	Natural mixed tocopherols	% oxygen	0.92	0.41	0.89	0.53	0.19	0.27
		Flavor	41	35 ^c	33 ^c	30 ^c	25 ^c

^a N indicates samples were nitrogen packed. Others were air packed.

^b Time when oxidized flavor first was noticed.

^c Metallic—not to be confused with oxidized or tallowy.

Vanilla as an antioxidant. In another study that was repeated three times with practically the same results, powdered cream-mixes containing NDGA and a pure 6-fold vanilla concentrate (made from Bourbon and Mexican beans) were compared as to keeping properties. The cream-mix had a composition of 30 per cent butterfat, 7.35 per cent milk solids-not-fat, 5 per cent sugar, and 0.2 per cent glycerol monostearate. The NDGA was added at two levels, 0.0025 per cent and 0.005 per cent, based on the

TABLE 3
Changes in oxygen concentration in head space gas and palatability of air-packed
and gas-packed dried cream-mix containing antioxidants

Batch no.	Antioxidant	Days storage at room temperature									
		7	30	60	92	120	152	182	213	265	365
23	% oxygen Flavor	20.61 41.5	20.05 38.0 ^b	19.40 36.0	19.16 35.0	18.44 33.0	17.14 33.0	17.11 33.0	15.95 31.0	12.45 20.0	2.12 20.0
23N ^a	% oxygen Flavor	1.66 43.0	1.63 39.0 ^b	1.11 39.0	1.37 38.0	0.77 41.0	0.30 40.5	0.65 39.5	0.52 38.0	0.89 36.0	0.21 35.0
24	0.0025% ^c NDGA	20.41 43.0	20.19 41.5 ^b	19.74 39.0	19.58 39.0	19.71 39.0	19.07 38.5	17.16 35.0	18.56 33.0	15.76 34.0	12.70 30.0
24N	0.0025% ^c NDGA	1.65 43.0	1.89 42.5	1.35 43.0	1.65 40.0 ^b	1.37 41.0	0.91 41.0	0.47 40.0	1.00 39.0	0.52 38.0	0.32 37.0
25	0.005% ^c NDGA	20.71 44.0	20.34 42.0	20.19 42.0	20.04 42.0	17.98 38.0 ^b	18.61 39.0	17.13 35.0	17.02 34.0	18.02 38.0	7.26 34.0
25N	0.005% ^c NDGA	2.22 44.0	2.22 44.0	1.45 43.0	2.05 42.0	1.11 40.0 ^b	0.72 41.5	1.23 40.5	1.17 39.5	0.49 40.0	0.15 37.0
26	0.1% pure vanilla conc.	21.04 44.0	20.41 44.0	19.96 44.0	19.93 43.0	19.71 42.0	19.13 41.0 ^b	18.69 37.0	18.40 37.0	15.54 39.0	11.58 34.0
26N	0.1% pure vanilla conc.	2.13 44.0	2.40 44.0	2.04 44.0	2.16 43.5	1.88 43.0	1.51 42.5	1.42 41.0 ^b	2.13 41.0	1.11 41.0	1.23 40.0

^a N indicates samples were nitrogen packed. Others were air packed.

^b Time when oxidized flavor first was noticed.

^c Based on per cent of fat.

weight of butterfat. It was added in 15 ml. of butter oil to the 60 per cent cream-mixes just before drying. To another mix, 0.1 per cent of pure vanilla extract was added just before drying. The data obtained during a year's storage at room temperature are given in table 3.

The antioxygenic properties of the vanilla were discovered when it was added to the cream-mix before drying to note whether the vanilla flavor was lost in the drying operation. This vanilla concentrate was added

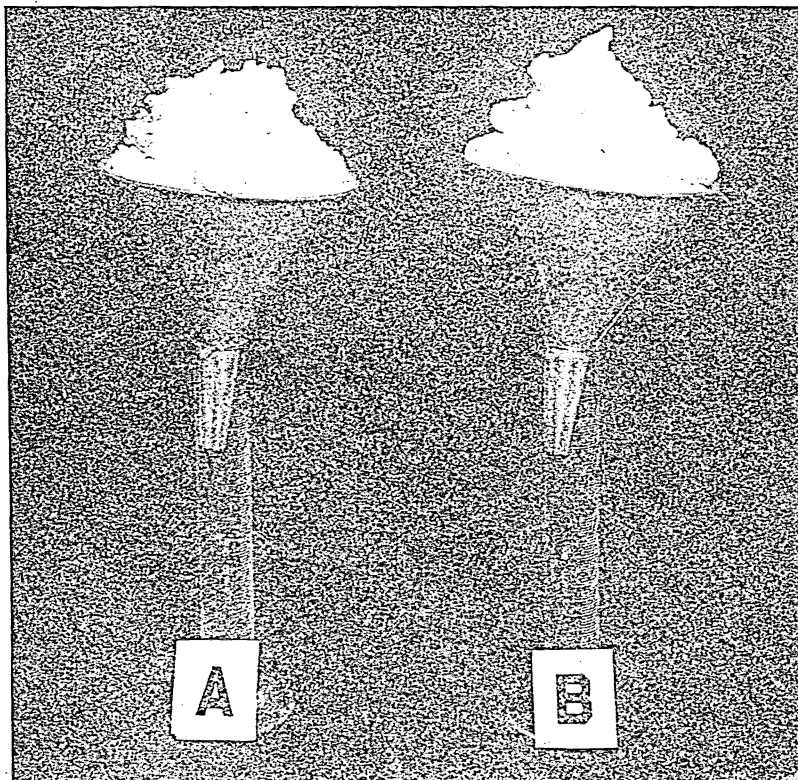


FIG. 1. Method used to measure drainage from whipped cream-mix. (A = Aerated cream made from fresh cream-mix (29.5% butterfat) B = Aerated cream made from reconstituted powdered cream-mix (29.5% butterfat). Both products made from same lot of cream.)

at the preheater just before spray drying the cream-mix at a temperature of 150° F. The processing or the drying operations did not seem to affect the intensity of the vanilla flavor of the reconstituted powdered cream-mix. The data indicate that this pure vanilla concentrate was a more effective antioxidant than NDGA. The nitrogen-packed control sample was oxidized at the 30-day examination period. The sample that contained 0.0025 per cent of NDGA was oxidized at 92 days of storage; when 0.005 per cent NDGA was added, the powdered cream-mix was oxidized at the

120-day examination. Nitrogen-packed powdered cream-mix containing 0.1 per cent of pure vanilla concentrate was not oxidized until examined at 182 days of storage, and then it was only slightly oxidized. Air-packed samples showed similar trends when stored with NDGA, and pure vanilla concentrate. At first the judges thought that the vanilla might be masking the oxidized flavor in the samples containing the vanilla. To check this possible masking, 0.1 per cent pure vanilla concentrate was added to a reconstituted control that was oxidized. The results obtained indicate

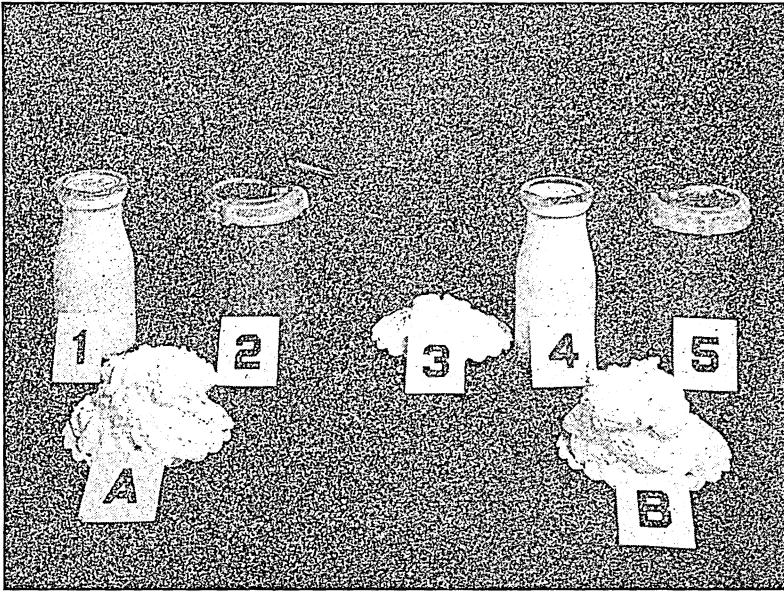


FIG. 2. Comparison of aerated cream made from fresh and reconstituted cream-mix (29.5% butterfat) (1 = Fresh cream-mix; 2 = charged container of fresh cream-mix; 3 = powdered cream-mix; 4 = reconstituted powdered cream-mix; 5 = charged container of reconstituted cream-mix; A = Aerated cream made from fresh cream-mix; B = Aerated cream made from reconstituted cream-mix. Both products made from same lot of cream).

that there was little, if any, masking of the oxidized flavor by the vanilla flavor.

The gas analysis data in table 3 indicate that when the vanilla concentrate was used, in general, there was more oxygen left in the headspace than in the headspace of the control or NDGA samples at the end of the storage period.

The chief constituent of vanilla beans from which vanilla extract is made is vanillin. Vanillin is a phenolic substance having the formula 4-hydroxy 3-methoxy benzaldehyde. Vanillin is the mono-methyl ether of protocatetheric aldehyde, the methoxy group being in the meta position to the aldehyde group. At low concentrations numerous phenolic substances

have the ability markedly to inhibit the autoxidation of fats. The most effective phenols are those which have some type of oxygen linkage in the ortho or para positions, or both, to the hydroxyl group. Some of the best known antioxidants of this type are hydroquinone, the tocopherols, gum guaiac and NDGA. The structural formula of vanillin is quite similar to other compounds that show antioxygenic properties. Consequently, it is possible the antioxidant properties of certain vanillas can be explained through their structural formulas.

Comparison of fresh cream-mix with reconstituted powdered cream-

TABLE 4
Butterfat, moisture, iron and copper content of powdered cream-mixes

Batch no.	Butterfat	Total solids	Moisture	Iron	Copper
	(%)	(%)	(%)	(p.p.m.)	(p.p.m.)
1	66.25	99.5	0.5	4.2	1.18
2	69.06	99.4	0.6	2.4	1.23
3	70.25	99.0	1.0	2.4	0.90
4	72.09	99.6	0.4	2.6	0.78
5	72.04	99.4	0.6	3.8	1.50
6	69.91	99.5	0.5	3.2	1.25
7	69.09	99.4	0.6	3.1	1.43
8	67.03	99.6	0.4	2.5	1.40
9	68.40	99.4	0.6	3.6	0.85
10	68.76	99.3	0.7	3.5	1.23
11	68.20	99.5	0.5	2.2	1.35
12	68.31	99.6	0.4	2.0	1.00
13	68.16	99.5	0.5	1.9	0.95
14	55.19	98.9	1.1	4.2	1.00
15	55.19	98.7	1.3	2.6	1.35
16	55.20	98.9	1.1	2.3	1.20
17	68.42	99.4	0.6	3.1	1.30
18	67.89	99.4	0.6	3.2	1.23
19	67.67	99.2	0.8	2.7	1.18
20	67.79	99.4	0.6	3.7	1.18
21	67.76	99.2	0.8	3.7	1.20
22	67.93	99.2	0.8	2.5	1.15
23	69.92	99.2	0.8	3.0	0.85
24	69.87	99.1	0.9	2.2	0.75
25	69.97	99.4	0.6	1.4	0.63
26	69.89	99.2	0.8	1.8	0.83
27	67.21	98.9	1.1	2.8	1.40

mix. To determine whether or not aerated cream made from powdered cream-mix is as satisfactory as aerated cream made from fresh cream-mix, a batch of cream-mix containing 29.5 per cent fat was divided into two lots. One lot was kept as fresh cream-mix. The other lot was dried in the usual manner and reconstituted with water to bring it back to its original composition of 29.5 per cent butterfat, 8 per cent milk solids-not-fat, 5 per cent sugar and 0.3 per cent emulsifying agent. Batch no. 27A, made from the fresh cream-mix, is exhibit A in figures 1 and 2 and batch no. 27, made from the reconstituted cream-mix, is exhibit B.

The only differences between the aerated cream-mix and the powdered

cream-mix were the dryness of the whip and the cooked flavor. The body and texture of the fresh product was firm and dry while the reconstituted product was firm but slightly moist. The flavor of the reconstituted product was slightly cooked.

Composition of powdered cream-mixes. Butterfat, moisture and iron and copper content were determined on all of the batches reported in this paper. Table 4 gives a summary of these data. The butterfat percentages of the dried cream-mixes recorded in the table vary somewhat due to variations in composition of the cream-mixes before drying. Batches 14, 15 and 16 were made with 19 per cent cream-mix. All other batches contained 29-30 per cent fat except batches 1, 2, 3 and 4, in which the butterfat was varied from 26 to 34 per cent. No difficulty was encountered in producing a cream-mix with low moisture content, since the dried product consisted of almost 70 per cent butterfat. The iron and copper contents of the dried cream-mixes are similar to the iron and copper contents of powdered whole milk made with the equipment used.

SUMMARY

Studies were made of variations in percentage of butterfat, milk solids-not-fat, and emulsifying agents in powdered cream-mixes. A product consisting of approximately 30 per cent butterfat, 7 to 8 per cent milk solids-not-fat, 5 per cent sugar, 0.3 per cent emulsifying agent and 0.1 per cent vanilla concentrate on a reconstituted basis gave satisfactory results. This cream-mix, when whipped by aeration, produced results similar to those obtained with the undried product.

There were no noticeable differences in whipping properties of reconstituted whipped cream-mix made from cream-mix sprayed with nozzle nos. 64, 72 and 79. The capacity of the drier was lowered by using nozzles with smaller orifices. The packing density was increased by using nozzles with larger orifices.

High spray pressures were found to be detrimental to the whipping properties of the reconstituted cream-mix, producing a product lacking in stability, containing large gas cells and having a moist appearance.

The keeping quality of the powdered cream-mix can be improved by packing in inert gas and by the addition of certain antioxidants before drying. As with powdered milk, it is desirable when storing the powdered cream-mix in inert gas to obtain a low oxygen content of the head-space on desorption. The antioxidants seemed to be more effective when the samples were packed in inert gas than in air. The most effective antioxidant was pure vanilla concentrate. Others that were effective were NDGA, gallic acid and Viobin.

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EFFECT OF DELAY IN DILUTING AND COOLING ON KEEPING QUALITY OF BULL SEMEN

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Technique used in the handling of bull semen and in the breeding of cows artificially has made great progress in the last decade. Even so, many problems still confront workers in artificial breeding. Although improved diluters are now in use and the cooling of semen to between 35 and 40° F. is almost universally practiced, very little experimental work has been done on the time effect of immediate or delayed dilution and cooling. Probably the standard practice is to collect the semen and try to hold it as near the ejaculation temperature as possible until returning to the laboratory, where cooling and diluting procedures are inaugurated. The time required for collecting two ejaculates from a bull and returning to the laboratory, of course, varies according to how far the bulls are from the laboratory and how quickly the ejaculates may be collected from each individual bull. In some cases this time may be negligible, but in other cases enough time undoubtedly is consumed that it may mean the difference between semen that would rate "good" and "poor".

Reports on actual experiments testing the effects of delay in diluting or cooling of semen have not been found. However, procedures described by various workers (2, 3, 5, 6, 7, 8, 11) indicate that diluting and cooling should immediately follow collection and that cooling should be done slowly. One report (4) gives evidence that it is not necessary to cool the semen slowly.

In Louisiana and other southern states the temperature shock to spermatozoa, particularly that due to cold weather, probably is not as important as it is in more northern climates. This study was conducted in an effort to determine if the keeping qualities of bull semen are affected by the time between ejaculation and dilution and the beginning of cooling.

EXPERIMENTAL PROCEDURE

Forty-two ejaculates from six different dairy bulls (five Holsteins and one Jersey) were used for this study. Following collection of each sample, three 1-ml. samples were taken from it for the experiment. These samples were treated as follows: No. 1, the diluting and cooling procedures were started immediately; no. 2, the semen was diluted immediately but the cool-

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ing procedure was not started until 45 minutes following collection; no. 3, both the diluting and the start of the cooling procedure were delayed for 45 minutes from time of collection.

The experiment was conducted between February 18 and April 29, 1947, inclusive. On the 11 actual experimental days during this period the air temperature as reported by the Baton Rouge weather bureau at 10:30 a.m. ranged from 44 to 80° F. and averaged 63.6° F.

The fresh semen was examined microscopically and rated into classes as described by Herman and Swanson (4), except that evaluations were made on the basis of 0.5 intervals. The same system of rating the samples was followed after storage at 35–40° F. for 24 and 72 hours. A variation of the methylene-blue test (1) was run on all samples, initially, and 24 and 72 hours after collection. One milliliter of fresh semen was mixed with 9 ml. of diluter. By using a small test tube, 1 ml. of methylene-blue solution was added to the diluted semen and mixed well. A 0.5-inch layer of mineral oil was added on top of the mixture to seal the tube, which then was placed in a water bath at 40° C. and the reduction time carefully noted. A variation of Beck and Salisbury's test (1), incubating the samples at 115° F. for 15 minutes and examining microscopically, was used on all samples 72 hours after collection.

The first 19 semen samples were diluted with synthetic pabulum (8) and the remaining 23 samples with yolk-citrate diluent (9). In no case were the two diluents used on portions of the same semen sample. All samples were diluted at the rate of 1:10, using 1 ml. of semen to 9 ml. of diluent, which first had been warmed to around 85 to 90° F.

A thermos bottle one-half full of water cooled to 35° F. was used when procedure called for the beginning of cooling immediately after collection. By slipping the vial of diluted semen partially through a hole in the thermos bottle stopper, gradual cooling was begun immediately. After returning to the laboratory, the temperature was taken and the vials of semen placed in 400-ml. beakers one-half full of water at a temperature the same as that of the semen. The beakers were placed in a refrigerator set at 35–40° F. and continued to be cooled until the desired storage temperature of about 38° F. was reached.

Where cooling was to be delayed 45 minutes—*e.g.*, until returning to the laboratory—the semen was diluted in a test tube and placed in 400-ml. beakers one-half full of water at about 85° F. The large beakers containing tubes of semen then were placed in a refrigerator and gradually cooled over a period of 2 to 2.5 hours to temperatures ranging from 35 and 40° F.

In analyzing the results of this study an analysis of variance (10) was run separately on: (a) Motility 24 hours after collection, (b) motility 72 hours after collection, (c) methylene-blue reduction time 24 hours

TABLE 1

*Comparison of motility ratings after storage for 24 and 72 hours between semen samples processed immediately and those delayed in diluting and cooling
(Mean of 42 samples)*

Procedures	Mean motility rating		
	After storage for 24 hr.	After storage for 72 hr.	72 hr. storage and incubation
No. 1, immediate diluting and cooling	3.13	2.20	1.33
No. 2, diluted immediately, cooling delayed 45 min.	3.11	2.04	1.12
No. 3, diluting and cooling delayed 45 min.	2.96	1.81	1.02

after collection, (d) methylene-blue reduction time 72 hours after collection, and (e) incubation test 72 hours after collection.

RESULTS

Motility ratings. Only slight differences in motility ratings (table 1) were noted between the averages for the three procedures based on observations made after 24 hours of storage. The mean value of 3.13 for no. 1 (immediate diluting and cooling) and 3.11 for no. 2 (immediate diluting and delayed cooling) samples indicated little advantage for immediate cooling. A greater effect was evidenced by the delay for 45 minutes in both diluting and cooling (no. 3), in which case the average was 2.96. Differences found after 24 hours of storage were highly significant statistically ($P < 0.001$), as were those found after 72 hours of storage.

Following 72 hours of storage, the average motility ratings (table 1) for the three procedures were 2.20, 2.04 and 1.81, respectively. These differences were greater than those found after 24 hours of storage and indicated that a delay in cooling or in both diluting and cooling tended to lower semen quality. This trend also is shown in the motility averages for

TABLE 2

*Comparison of methylene-blue reduction time of semen samples treated differently following collection
(Mean of 42 samples)*

Procedures	Mean reduction time	
	After storage for 24 hr.	After storage for 72 hr.
	(min.)	(min.)
No. 1, immediate diluting and cooling	15.76	19.21
No. 2, diluted immediately, cooling delayed 45 minutes ...	16.91	20.14
No. 3, diluting and cooling delayed 45 minutes	17.50	22.88

samples stored for 72 hours and then subjected to incubation at 115° F. for 15 minutes prior to examination. These averaged 1.33, 1.12 and 1.02, respectively, for the three procedures.

Methylene-blue reduction time. As in motility ratings, the time required to reduce methylene blue (table 2) likewise indicated advantages for the immediate dilution and cooling of the semen samples. After storage for 24 hours the reduction time for the three procedures was 15.76, 16.91 and 17.50 minutes and after 72 hours of storage 19.21, 20.14 and 22.88 minutes, respectively. While the trend was the same for the tests made at the two periods of time, only those differences found for the 24-hour period proved to be statistically significant ($P < 0.001$).

SUMMARY

Forty-two ejaculates from 6 different bulls were used in an experiment to determine if the amount of time required to collect semen and get it to the laboratory before dilution and beginning of cooling would affect the keeping qualities of the semen.

Using motility ratings from microscopic examinations made after 24 and 72 hours of storage before and after incubation and methylene-blue reduction tests as criteria of semen quality, it was found that the immediate processing of semen following collection was desirable for the maintenance of desirable characteristics of semen. Delay in either the diluting or the start of the cooling process tended to lower ratings made of the semen.

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EFFECT OF FEEDING TOCOPHEROLS TO DAIRY COWS ON THE QUANTITY AND THE FAT CONTENT OF MILK PRODUCED¹

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Preliminary results obtained in an experiment designed to determine the effect of feeding mixed tocopherols to dairy cows on their milk and butterfat production do not agree with those presented recently by Harris *et al.* (1). These workers reported that the feeding of mixed tocopherols at the rate of 1.0 g. daily per cow brought about an increase of about 27 per cent in the fat concentration and 21 per cent in the total quantity of milk (4 per cent fat-corrected) produced. Their experiment, however, was carried on in a privately owned herd and the milk was tested for butterfat content on only 2 days each month.

EXPERIMENTAL

Seventeen purebred cows, ten Jerseys and seven Holsteins, of various ages were used in the present study. They were divided into two groups with eight cows in the control and nine in the supplemented group. The breed and age of cows used in both groups and their further division into classes A, B and C according to dates of calving are indicated in table 1.

The experiment started with a 10-day preliminary period, which ended May 21, 1947. During this period, none of the cows in either group was fed the tocopherol supplement. Feeding of the supplement, known as "Myvadry", to cows in the supplemented group was started on the evening of May 21 and continued through July 5, 1947. As in the experiment of Harris *et al.* (1), the supplement was added to the grain ration at time of feeding, in such amounts as to provide each cow with 1.0 g. of mixed tocopherols daily.

Cows in both groups were fed and managed alike except for the supplement. All cows were fed normal herd rations, which included good quality alfalfa hay and corn silage, along with concentrates in amounts according to their milk production. The Jerseys were fed grain at the rate of 1 lb. to every 2.5 to 3.5 lb. of milk produced and the Holsteins 1 lb. to every 4 to 5 lb. of milk produced daily. The grain mixture contained 225 lb. ground corn, 300 lb. ground oats, 300 lb. ground barley, 75 lb. linseed meal, 75 lb. soybean oil meal, 10 lb. steamed bone meal, and 15 lb. salt. When weather permitted, cows were kept outdoors except when being fed. Beginning on June 5, all the cows in both groups were turned on pasture

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daily but they continued to receive other roughage and the usual amounts of grain.

Records were kept of the amount and the fat content of milk produced daily by each cow during the entire period of the experiment, including the 7 days after feeding of the supplement had been discontinued. The Babcock method was used in determining the per cent of fat in the milk. Table 2 shows the average per cent of fat in the milk and the calculated average daily production per cow in pounds of 4 per cent fat-corrected milk of cows in each class and group during the preliminary period and at

TABLE 1
Breed, date of birth and date of calving of cows used in the experiment

Animal no.	Breed	Class ^a	Date of birth	Date of calving
Control group				
256	Jersey	A	1-24-39	3-28-47
295	"	A	12-20-42	4- 6-47
302	"	A	2-27-44	3-28-47
275	Jersey	B	7- 2-40	12-26-46
299	"	B	11-10-43	12-23-46
474	Holstein	C	12-23-37	2-20-47
813	"	C	4- 8-41	1-13-47
838	"	C	11-17-43	1-15-47
Supplemented group				
282	Jersey	A	9-20-41	4-12-47
300	"	A	12-18-43	4- 1-47
267	Jersey	B	12-15-39	1-31-47
280	"	B	6-26-41	1-23-47
297	"	B	2-24-43	1- 6-47
480	Holstein	C	3- 2-38	2- 3-47
492	"	C	11-21-39	1- 5-47
814	"	C	5-14-41	12-25-46
835	"	C	5-25-43	2-27-47

^a The calving dates of cows in Classes A, B and C in the control group are approximately the same as of those in the corresponding classes in the supplemented group.

5-day intervals while supplement was fed and also during the 7-day period after feeding of supplement was discontinued.

The condition, general appearance and appetite of all animals remained good throughout the entire period of the experiment, with no class or group showing any superiority in any respect. The data in table 2 fail to indicate any tendency of a rise in the fat content of the milk from the cows in the supplemented group after feeding of the tocopherol was started on the evening of May 21; neither is there any evidence of a drop in the fat percentage during the 7 days of observation after feeding of the supplement was discontinued on July 6. Likewise, the supplementation had no

TABLE 2
Average per cent of fat in milk produced and daily milk production (4% fat-corrected milk) of cows by classes
and by entire groups during the indicated periods^a

Group	Class	May			June							July	
		12-21	22-26	27-31	1-5	6-10	11-15	16-20	21-25	26-30	1-5	6-12	
% of fat in milk													
Control Supplemented	A	4.73	4.73	4.52	4.60	4.97	5.09	4.75	4.80	4.69	4.60	4.72	
	A	4.64	4.37	4.49	4.37	4.74	5.00	4.61	4.61	4.50	4.64	4.48	
	B	5.13	5.21	5.32	5.23	5.63	5.58	5.58	5.49	5.18	5.38	5.37	
	B	4.88	4.64	4.82	4.81	5.18	5.25	5.07	4.94	4.86	4.96	4.99	
	C	3.12	3.07	3.12	3.12	3.13	3.23	3.04	2.96	2.95	3.09	3.04	
	C	3.15	3.22	3.18	3.24	3.20	3.26	3.21	3.10	2.92	3.17	2.95	
	All	4.14	4.10	4.07	4.09	4.27	4.37	4.13	4.11	4.00	4.09	4.10	
	Supplemented	All	3.87	3.86	3.93	3.90	4.10	4.18	4.03	3.93	3.79	3.98	3.84
lb. 4% fat-corrected milk													
Control Supplemented	A	31.8	29.8	28.0	26.9	30.8	30.3	28.7	28.7	26.2	24.4	25.1	
	A	37.6	36.8	35.9	35.0	38.6	38.7	36.5	34.1	30.5	28.4	28.9	
	B	21.4	19.8	20.0	19.7	22.1	22.5	21.2	21.4	18.3	19.9	18.2	
	B	21.7	21.4	20.0	20.0	23.4	23.3	22.6	22.4	20.4	20.2	20.4	
	C	25.0	24.5	23.5	23.5	26.4	26.6	25.6	24.7	22.8	22.2	21.9	
	C	28.6	26.3	24.9	26.5	26.9	28.5	26.6	26.7	23.9	23.3	22.4	
	All	26.6	25.3	24.3	23.8	27.0	27.0	25.7	25.4	22.9	22.6	22.3	
	Supplemented	All	28.2	26.3	25.7	26.2	28.4	29.0	26.9	24.2	23.6	23.3	

^a None of the cows in either group was fed the tocopherol supplement during the first and the last periods indicated.

apparent effect on the quantity of milk produced, as expressed in terms of 4 per cent fat-corrected milk. Although the cows in the supplemented group produced at a somewhat higher level than those in the control group, this difference was as great during the preliminary period as it was later during the time when the supplement was fed.

Both the per cent of fat in the milk and the amounts of 4 per cent fat-corrected milk produced by cows in each class and group varied widely from period to period during the progress of the experiment. These variations, however, are no more marked in one class or group than in the other; furthermore, they show a definite tendency to occur simultaneously in the several classes and groups as though resulting from a common cause. Such an effect is indicated by the marked increase in milk production and to a less marked degree by the rise in the per cent of fat in the milk produced by cows in all classes of both groups after they were turned on pasture on June 5.

A more comprehensive study of this problem is under way, the results of which will be presented at some later date.

SUMMARY AND CONCLUSIONS

A feeding trial was conducted to determine the effect of supplementing the rations of lactating dairy cows with 1 g. of mixed tocopherols daily per cow on the amount and fat content of the milk produced. Seventeen cows were used, eight of which received basal ration and the remainder received supplemental tocopherol daily from May 21 to July 5, inclusive. Milk from each cow in both groups was weighed and tested for butterfat content daily.

Neither the amount nor the fat content of the milk produced appeared to be affected by feeding of the tocopherol supplement. Such supplementation produced no changes in the appearances and appetites of the animals.

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THE ELIMINATION OF INTERFERING SUBSTANCES IN THE KAY-GRAHAM PHOSPHATASE TEST WHEN USED FOR HARD RIPENED CHEESE¹

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The Kay-Graham phosphatase test (5), including the Gilcreas and Davis modification (4), is not suitable for the analysis of hard ripened cheese. A recent report by Gilcreas (3) comparing the effectiveness of several phosphatase tests in the examination of hard cheese emphasized this point when he stated the following concerning the Kay-Graham method: "... the control values particularly in the examination of aged cheese are so high as to limit sharply the utility of this test for detecting the presence of the active enzymes in the sample. This interference is undoubtedly caused by amino acids, particularly tyrosine which is always present in aged cheese." Other phosphatase tests such as those of Sanders and Sager (8) and Scharer (9) would not encounter this type of interference as 2,6-dibromo-quinone-chloroimide (BQC), the color reagent used for these tests, is specific for phenol, whereas the Folin-Ciocalteu color reagent used in the Kay-Graham test originally was developed to determine tyrosine and tryptophane (2).

Results from recent investigations upon the protein decomposition products of cheese (1, 6) encouraged the authors to believe that all the interfering substances could be eliminated in the application of the Kay-Graham test to cheese. It was obvious that tyrosine and possibly tryptophane are interfering substances and that the amine, tyramine, which was shown (6) to be present in all cured commercial Cheddar cheese, also contributed greatly to the interference. This amine is water soluble and forms a blue compound with the Folin-Ciocalteu reagent. In the standard Kay-Graham method it passes into the filtrate to intensify the color.

To eliminate all interfering amino acids and amines from the Kay-Graham test, certain solubility principles were considered. Tyrosine and tryptophane, as well as most of the other amino acids, are insoluble in ether. Tyramine is soluble in ether when extracted under slightly alkaline conditions, but it is insoluble in ether under acid conditions. Phenol, on the other hand, is very soluble in ether under acid conditions.

EXPERIMENTAL RESULTS

Utilizing the solubility characteristics of the various compounds in-

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volved, the following approach, designated as the trichloroacetic acid technique, was evolved.

The method. A sample of ground cheese (0.5 g.) was incubated with 10 ml. of buffer substrate and two drops of chloroform for 24 hours at 37° C. The buffer substrate contained 1.09 g. disodium phenyl phosphate and 17.54 g. of sodium barbital per liter of water, to which 10 ml. of chloroform were added. Following this incubation, 1 ml. of a 25 per cent trichloroacetic acid solution was added. The resulting precipitate was filtered off through Whatman no. 42 filter paper. Five milliliters of the clear filtrate then were pipetted into a standard Mojonnier extraction flask. Enough 1 per cent hydrochloric acid solution was added to this flask to bring the liquid to the bottom of the neck of the flask. Then 25 ml. of ethyl ether were added. The Mojonnier flask was stoppered with a cork covered with tinfoil and inverted slowly 20 times. After the agitation was completed, the clear ether was poured off into a large tube containing 5 ml. of distilled water. The ether was boiled off in about 5 minutes by immersing the tube in a beaker of hot water.

To the 5 ml. of remaining aqueous solution, 2 ml. of Folin-Ciocalteu reagent (diluted 1 to 2) were added, followed immediately by 2 ml. of 14 per cent sodium carbonate. The mixture was placed in boiling water for 5 minutes, after which it was cooled, filtered and the color readings taken. A Luximeter colorimeter was used throughout the entire experimental work.

Phosphatase and interfering blank values of Cheddar cheese. Data were obtained on an assorted group of 25 representative commercial Cheddar cheeses. In table 1, columns 4 and 5, the phosphatase values and interfering blank values of the different cheeses are shown as obtained by the trichloroacetic acid technique. The interfering blanks were obtained by using a plain buffer solution without the disodium phenyl phosphate substrate and by eliminating the incubation period which showed the possibility of removing interfering substances present in the original cheese. Otherwise the method was similar. Controls also were run on many of the cheeses. In column 6, table 1, are shown the interfering blank values obtained on the cheeses by the standard method of Kay-Graham (4, 5) using a 0.5-g. sample of cheese.

The phosphatase values obtained by the trichloroacetic acid technique show that a wide variation existed among these cheeses. Some of these tested cheeses were made from raw milk and some were made from pasteurized milk. The important fact is that all the cheeses tested, ranging in age from 7 months to 10 years, showed interfering blank values which were extremely low, varying from 0.002 to 0.009 mg. phenol per 0.5 g. of cheese and averaging 0.004 mg. per 0.5 g. of cheese. Control values on the cheeses tested also were extremely low. The final filtrates in the trichloroacetic

technic always were clear blue, being very similar in character to those obtained on milk. It was very easy to read intensity differences.

The interfering blank values obtained using the standard Kay-Graham method (table 1), with one exception, were all extremely high, indicating gross contamination by interfering substances produced during ripening.

TABLE 1

The phosphatase and interfering blank values of 25 commercial Cheddar cheeses obtained by the trichloroacetic acid modification of the Kay-Graham method, the Standard Kay-Graham method, and the Sanders-Sager method

Cheese			Trichloroacetic acid technic		Standard Kay-Graham method	Sanders-Sager method ^a
No.	Age	Mfgs. report	Phosphatase values	Interfering blank values ^b	Interfering blank values ^b	Phosphatase values
	(mo.)		(mg. phenol/0.5 g. cheese)		(mg. phenol/0.5 g. cheese)	(γ phenol/0.25 g. cheese)
1	8	Past.	0.013	0.004	0.144	2.5
2	19	Past.	0.010	0.006	0.265	3.0
3	8	Past.	0.014	0.003	0.188	3.0
4	17	Past.	0.012	0.005	0.302	1.0
5	18	Past.	0.013	0.002	0.271	2.0
6	10	Past.	0.004	0.002	0.360	2.0
7	8	Past.	0.020	0.002	0.225	5.0
8	11.5	Past.	0.046	0.004	0.200	6.0
9	8	Past.	0.045	0.005	0.122	6.0
10	13	Past.	0.060	0.004	0.350	9.0
11	14	Raw	0.698	0.003	0.331	35.0
12	126	Raw	0.785	0.007	0.703	> 40.0
13	8.5	Raw	1.226	0.006	0.241	>> 40.0
14	16	Raw	0.821	0.004	0.382	> 40.0
15	15	Raw	0.769	0.006	0.422	> 40.0
16	38	Raw	0.745	0.006	0.466	30.0
17	7	Raw	0.849	0.004	0.181	> 40.0
18	41	Raw	0.702	0.003	0.703	40.0
19	35.5	Raw	0.978	0.006	0.497	> 40.0
20	13.5	Raw	0.773	0.006	0.125	> 40.0
21	8	Raw	0.853	0.002	0.038	>> 40.0
22	7	Raw	0.637	0.003	0.166	> 40.0
23	8	Raw	0.853	0.009	0.196	> 40.0
24	29	Raw	0.749	0.009	0.396	35.0
25	16	Raw	0.702	0.004	0.125	> 40.0

^a A value of over 3.0 for the Sanders-Sager method indicates cheese made from improperly pasteurized milk.

^b Shows amount of interfering substances developed in cheese during ripening and not removed by the trichloroacetic acid technic.

The range extended from 0.038 to 0.703 mg. phenol per 0.5 g. cheese. In cheese of low phosphatase values, the interfering blank values often were as much as 60 times as great as the actual phosphatase value of the cheese.

Although it was evident that the interfering substances could be eliminated completely, there was as yet no evidence to show that by using this

technic it would be possible to follow differences in the phosphatase concentration of various cheeses comparable to those shown by standard phosphatase methods. To check this point, all the cheeses were tested by the phosphatase method of Sanders and Sager (8) and these results were compared to results using the new technic, even though there is now no intent of presenting a final procedure for the new technic. The dilution procedure for the Sanders-Sager method which would make the results of those cheeses containing more than 40 γ of phenol per 0.25 g. cheese more quantitative was omitted although this omission does not prevent a correct interpretation of these results. The data, presented in table 1, include the heat treatment history of the cheese milk and the age of the various cheeses, both as given by the manufacturers. It was evident from the results obtained by the Sanders-Sager phosphatase test that some of the cheeses, stated to have been made from pasteurized milk, actually were made from underpasteurized milk. Although at the present time data are not available to show at what concentration of phenol the trichloroacetic acid technic distinguishes raw from pasteurized milk cheese, it is encouraging to note that where there are phenol-value increases in the Sanders-Sager method (table 1), there also are phenol-value increases in the trichloroacetic acid modification (column 4, table 1) of the Kay-Graham method, although not necessarily of the same magnitude. With a few exceptions, the phenol values of the cheese were related to the reported pasteurization of the milk.

The magnitude of the phenol values for cheese obtained by the trichloroacetic acid technic corresponds relatively closely to that obtained on milk with the standard Kay-Graham method, although the critical value dividing the raw from the pasteurized product does not appear to be quite the same.

Interfering substances other than tyrosine and tyramine. It has been reported by Leahy *et al.* (7) that the Folin-Ciocalteu reagent also gave strong colors with a variety of chemical compounds including diacetyl, acetylmethylcarbinol, cystine, l-leucine, indole, uric acid, allantoin and guanine. Although none of these compounds has been reported in cheese in significant amounts, it may be well to point out that most of them are insoluble in ether and others are insoluble in aqueous solutions. However, diacetyl is considered to be soluble in both ether and water. Although only traces have ever been reported in cheese, a study was made to note the effect of the addition of small amounts to cheese. The amounts selected were one to ten times that usually found in ripened butter, which contains on the average of about 3-4 p.p.m. No significant increase was noted in the final readings after these additions, indicating even if diacetyl were ever present it would not affect the final results. The very fact that it was possible to test 25 cheeses of vastly different history and obtain low blanks is another indication that these interfering substances are no longer significant using the modified method.

DISCUSSION

The Kay-Graham phosphatase test as commonly used for milk has been found inaccurate on ripened hard cheeses because of the non-specificity of the Folin-Ciocalteu reagent toward phenol. As the Kay-Graham test is so valuable in the dairy industry, any attempt to overcome its inaccuracy on cheese should be encouraged. Although at this time no attempt has been made to present a routine method for the Kay-Graham phosphatase test on cheese, the removal of the interfering substances should stimulate development of such a method. Additional information is being gathered to perfect the details of the new technic and to show the adaptability and sensitivity of this technic in distinguishing cheeses made from milk heated at different times and temperatures.

The principle employed for the elimination of interfering substances actually consisted of producing conditions which allowed for the selective removal of most of the free phenol produced. The free phenol was produced first in an alkaline medium by the phosphatase enzymes and then extracted with ether under acid conditions so that none of the amino acids or amines likely to produce interference would be extracted. Removal of free phenol by washing with ether is a common practice in the purification of some chemical reagents and biological materials containing phenolic substances. It then was possible to retain the phenol in an aqueous solution by boiling off the ether. Because of its high boiling point (182° C.), probably very little phenol would be lost.

There is no doubt that tyramine is a very important factor contributing to the blue interference, as every cheese in this series contained it in some concentration. If any other products derived from the amino acids or other chemical substances were responsible for interference, they also are either insoluble, as almost negative interfering blanks and controls were obtained using the trichloroacetic technic.

The use of trichloroacetic acid was found very desirable because it possesses flocculating properties without requiring the aid of heat. It also aided in better color development for some unknown reason. In addition, a clear filtrate always was obtained prior to extraction. However, trichloroacetic acid is soluble in ether and in order to keep the filtrate at pH 1-2 during the extraction, an inorganic acid was added.

Occasionally an emulsion was encountered in the extraction process. This easily was broken by running hot tap water over the lower chamber of the Mojonnier flask for about 20 seconds with the cork loosely stoppered, followed by rapid cooling under the cold water tap.

The standard Kay-Graham method for milk expresses the results in units of *mg. phenol per 0.5 ml. milk* and uses only an aliquot portion of 0.5-ml. milk in the test. In the interests of uniformity the unit used for the trichloroacetic acid modification is *mg. phenol/0.5 g. cheese* even though

here again an aliquot sample smaller than 0.5 g. cheese is used for the final color determinations. For basic quantitative measurement of phenol in certain studies all dilutions should be considered. This can be done easily in this modification, if desired, but in the case of the phosphatase test where the method is mainly empirical in nature this is not considered essential.

SUMMARY

A technic was developed for the selective separation of free phenol from interfering substances in the Kay-Graham phosphatase test when used on hard ripened cheese. Tyrosine and tyramine were important interfering substances.

The elimination of the interfering substances was accomplished by using trichloroacetic acid as a precipitant for the cheese proteins, and by extracting the free phenol, formed as a result of phosphatase activity in an alkaline substrate, with ether under acid conditions using a Mojonnier-type extractor. The ether containing the phenol was placed in distilled water and then was boiled off. The aqueous solution then was treated with the Folin-Ciocalteu reagent and the amount of phenol determined with a colorimeter.

The amount of interfering substances in ripened cheese not removed by using the new trichloroacetic acid technic was extremely low, as values averaging 0.004 mg. phenol per 0.5 g. cheese were obtained for 25 cheeses varying in age from 7 months to 10 years.

Phenol values obtained on cheese using the new technic showed changes in phosphatase activity which corresponded well with results obtained on the same cheeses with the Sanders-Sager phosphatase method. The phenol values obtained using this new technic on cheese were on the order of those obtained on milk using the standard Kay-Graham method, but no complete data have been obtained yet to establish the final details of the test and to show at what point the new technic would distinguish raw from pasteurized milk cheese.

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DEHYDRATED SWEET POTATOES AS A CONCENTRATE FEED FOR DAIRY CATTLE¹

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Selection of the best quality sweet potatoes for table use yields culls which may be dehydrated and used for animal feed. High yields of sweet potatoes in some areas may justify growing them specifically for animal feeding, especially where corn yields are low. The present study was undertaken to determine the value of dehydrated sweet potatoes as a concentrate feed for dairy cattle.

REVIEW OF LITERATURE

Feeding trials conducted with fattening pigs (9, 10, 18) indicated that dehydrated sweet potatoes did not produce satisfactory gains, largely due to low palatability and a laxative effect.

For fattening steers, more satisfactory results have been obtained. When sweet potatoes replaced all of the corn in the ration, gains were less rapid than on corn (10, 11, 17) and were less efficiently made (10, 11). In a mixed ration, sweet potatoes were found equal to corn except for the lower protein content (7). In comparison with corn and wheat, gains were as rapid on sweet potato rations but the appraised value was slightly lower (6). When replacing only 50 per cent of the corn, sweet potatoes gave more rapid gains and the selling price on the steers was higher (17).

For dairy cows, Copeland (5) found that dried sweet potatoes were 90.75 per cent as valuable as corn for milk production but that the butter from sweet potato-fed cows had 37.98 I.U. per g. of vitamin A whereas that from the corn-fed cows contained only 31.11 I.U. per g. Trials with dairy cows in Louisiana (14, 16) showed that dehydrated sweet potatoes have approximately 88 per cent of the value of yellow corn meal, but are approximately 17 per cent more valuable than ground snapped corn, including cob and shuck.

Digestion trials on dehydrated sweet potatoes have been carried out with steers and lambs (3) and with dairy cows (14, 16). Briggs *et al.* (3) found that with steers the digestibility of the nitrogen-free extract of sweet potatoes was 93.4 per cent when fed with prairie hay and cottonseed meal and 98.5 per cent when fed with alfalfa hay. With lambs the correspond-

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ing values were 87.8 and 92.4 per cent. On a dry matter basis, the total digestible nutrients ranged from 78.7 to 91.4 per cent.

Rusoff *et al.* (14) and Seath *et al.* (16) had difficulty in obtaining the apparent digestibility of fat, fiber, and protein in dehydrated sweet potatoes, but in four trials found digestibility coefficients of 83.47 to 94.46 per cent for the nitrogen-free extract, and calculated total digestible nutrient values from 71.78 to 81.06 per cent on the dry basis. The lower values were for off-grade sweet potatoes which had been cut and bruised and heated to a higher temperature than commonly used.

EXPERIMENTAL PROCEDURE

In this experiment two methods were used to determine the feeding value of dehydrated sweet potatoes. First, a 75-day trial was conducted to determine the milk-producing properties relative to ground yellow corn. Secondly, a digestion trial was conducted to determine digestibility of the main components in order to estimate the total digestible nutrients for more direct comparison with other carbohydrate feeds.

Milk production trial. Four groups of three Holstein cows each were used in the milk production trial. The cows had been fresh from 41 to 142 days at the beginning of the trial; the cow which had been fresh for only 41 days had reached her peak production. These were grouped according to age (six mature cows and six first-calf heifers) and according to milk production within the age groups. Three rations were fed as indicated below. After an equalization and standardization period of 2 weeks, the cows were placed on the experiment, which consisted of three periods of 25 days each, of which the first 5 days was a transition period and the remaining 20 days the experimental period.

The animals were fed alfalfa hay and corn silage at a rate of approximately 8 lb. of hay and 24 lb. of silage per 1,000 lb. of body weight. Grain was fed at first at rates based on the grain feeding table (Appendix Table IXa) in *Feeds and Feeding* (13) for cows receiving the 1.5-lb. hay equivalent, but was adjusted so as to maintain production at approximately the level previous to adjustment. More grain was allowed those animals in their first lactation to allow for growth requirements. Concentrate allowances ranged from 11.6 to 21.0 lb. per day.

Following the suggestion of Lucas (12), the amount of concentrates for all cows was reduced at a rate uniform for all animals regardless of the ration received. As the decrease in production was very slight after the first 2 weeks, no adjustment was made until the end of the first feeding period, at which time the concentrate allowance was reduced 1.5 per cent. The same reduction was made at the end of the second period.

Three rations were used. Ration A consisted of ground shelled corn and soybean meal in a ratio of 650 lb. ground corn to 125 lb. soybean meal.

Ration *B* consisted of 325 lb. ground corn, 325 lb. dehydrated sweet potatoes and 125 lb. soybean meal. Ration *C* consisted of 650 lb. dehydrated sweet potatoes plus 125 lb. soybean meal. This proportion of soybean meal was calculated as supplying sufficient protein to the cows on ration *C*, and some excess to those on rations *A* and *B*. Because of some difficulty in grinding the dehydrated sweet potatoes with the high moisture content when received, the sweet potatoes were fed as they came from the bag, in shreds about 0.25-inch square by 1 to 2 inches long. For cows on ration *B* the sweet potatoes were weighed separately, and the corn and soybean meal mixed in the proper proportion were weighed separately. For cows on ration *C*, the sweet potatoes and soybean meal were weighed separately.

The sweet potatoes used in dehydration were principally the Maryland Golden variety, but small amounts of Jersey-type sweet potatoes were mixed with them. They consisted of the culls, such as the jumbos, small sized, cut and bruised sweet potatoes. All were cleaned and free of decay.

The ration sequences were arranged according to the method of Cochran

TABLE 1
Organization of milk production trial showing ration sequences

Period	Groups I and III			Groups II and IV		
	Cow 1	Cow 2	Cow 3	Cow 1	Cow 2	Cow 3
I	A	B	C	A	B	C
II	B	C	A	C	A	B
III	C	A	B	B	C	A

et al. (4) to allow for measurement of carry-over effects if these should persist beyond the 5-day transition period. The sequences were arranged as shown in table 1.

Within each group, the particular sequences were assigned to the cows at random.

Milk weights were recorded for each milking. A 1-day composite was accurately prepared once in each 5-day subperiod for a Babcock test. On the last day of each main period a carefully composited sample of milk was prepared for butterfat test, carotene and vitamin A analysis. For the carotene and vitamin A analysis, the method of Boyer *et al.* (2) was used with slight modification. After the extract was concentrated, it was passed through a small chromatograph of sodium carbonate (Frank W. Kerr Co., Detroit, Mich.). Carotene and vitamin A then were determined as recommended. At the same time, blood samples were drawn and the plasma analyzed for carotene and vitamin A according to the method of Boyer *et al.* (1), also with slight modifications. Only 5 ml. of plasma were extracted. After precipitation of the carotene-fat mixture, the samples were stored in the refrigerator until the precipitate had clumped, to facilitate filtration. This storage has not been found to reduce the vitamin A.

Body weights were determined at the end of each period by weighing on three successive days.

Digestion trial. Four mature Holstein cows nearing the end of their lactation periods were dried off 3 to 4 months before they were due to calve. Their requirements for maintenance were estimated according to the Morrison standard, with no special allowance for gestation. The amounts of mixed timothy-clover hay necessary to meet these requirements then were calculated. Two of the cows were placed on the hay ration exclusively. The other two cows received one-half of the calculated amount of hay and an equal number of pounds of dehydrated sweet potatoes.

After 7 days on these rations, the cows were placed in the digestion stall room for the digestion trial carried out as described by Eheart *et al.* (8).

TABLE 2
*Analysis of variance of fat-corrected milk, average butterfat test,
and body weight per period per cow*

Source	Fat-corrected milk		Butterfat test		Body weight	
	Degrees freedom	Mean square	Degrees freedom	Mean square	Degrees freedom	Mean square
Period	2	3,395	2	0.120*	2	2,249**
Group	3	122,416**	3	0.113*	3	47,101**
Period \times group	6	4,202*	6	0.070	6	821*
Cows within groups	8	9,905*	8	0.196**	8	26,627**
Ration	2	0.045	2	1,599*
Direct (adjusted)	2	14,180**
Residual (adjusted)	2	774
Error	12	1,248	14	0.029	14	261
Standard error per cow	35.3 lb.	0.170%	16.2 lb.
Coefficient of variation (%)	4.78	5.00	1.44

* Represents significance at the 5% point.

** Represents significance at the 1% point.

At the end of this period they were removed from the digestion stalls and changed to the other ration; those which had received hay only were cut to one-half the amount of hay and given an equal amount of sweet potatoes. Those which had been receiving hay and sweet potatoes were changed to hay only, in an amount equaling the total feed received previously. After 9 days the cows were returned to the digestion stalls for a second digestion trial. The changes in weight of the animals themselves were small and not significant.

RESULTS

Milk production trial. The results of the analysis of variance of the data obtained on 4 per cent fat-corrected milk, butterfat test, body weight, blood plasma carotene, blood plasma vitamin A, milk carotene and milk vitamin A are presented in tables 2 and 3. This analysis follows that of

TABLE 3

Analysis of variance of blood plasma carotene, blood plasma vitamin A, milk carotene, and milk vitamin A (γ /100 ml.)

Source	Degrees of freedom	Mean square			
		Plasma carotene	Plasma vitamin A	Milk carotene	Milk vitamin A
Period	2	16,088**	80.44**	62.92**	793.29**
Group	3	5,919*	69.47**	24.03	17.78
Period \times group	6	1,940	4.32	43.26*	9.75
Cows within groups	8	8,716**	39.59**	17.15	18.00
Ration					
Direct (adjusted)	2	33,806**	138.12**	111.52**	60.06*
Residual (adjusted)	2	507	10.60	2.86	0.38
Error	12	1,483	7.43	9.96	10.28
Standard error per cow		38.5	2.73	3.16	3.21
Coefficient of variation (%)		15.9	10.7	19.0	19.3

* Represents significance at the 5% point.

** Represents significance at the 1% point.

Cochran *et al.* (4) with the analysis for direct and carry-over or residual effects of the rations except in the case of butterfat test and body weight. These latter items were analyzed according to the usual procedure without breakdown into direct and residual effects. In no case did the residual effects even approach significance. Direct ration effects were significant at the 1 per cent point for fat-corrected milk, blood plasma carotene, blood plasma vitamin A and milk carotene. Direct ration effects were significant at the 5 per cent point for milk vitamin A. Ration effects were significant at the 5 per cent point for body weight but were not significant for the butterfat test.

Because the residual effects were slight and not statistically significant, the mean values actually obtained, without adjustment, are presented. The mean values for the three rations are presented in table 4 with percentage relationships based on ration A as 100.

TABLE 4

Mean values obtained for rations A, B, and C, and percentage relationships based on ration A as 100

	Mean values			Percentage of ration A	
	Ration A	Ration B	Ration C	Ration B	Ration C
4% fat-corrected milks	38.4	37.4	35.1	97.4	91.4
Butterfat test (%)	3.44	3.42	3.34	99.4	97.1
Body weight (lb.)	1115.3	1118.5	1136.7	100.3	101.9
Plasma carotene (γ /100 ml.)	170.3	277.2	279.8	162.8	164.3
Plasma vitamin A (γ /100 ml.)	21.8	26.1	28.5	119.7	130.7
Milk carotene (γ /100 ml.)	13.0	16.9	20.0	130.0	153.8
Milk vitamin A (γ /100 ml.)	13.9	17.0	19.0	122.3	136.7

^a Lb. per cow per day.

Digestion trial. The digestion coefficients and their standard errors and also the average of seven analyses of the components of the dehydrated sweet potatoes used in the trial are given in table 5.

On the basis of the analysis of dehydrated sweet potatoes actually used in the digestion trials, and considering negative digestion coefficients as zero, the total digestible nutrients are 79.0 per cent on a dry matter basis or 69.6 per cent on a 12 per cent-moisture basis. Using the average of the seven analyses as presented in table 5, the total digestible nutrients are 80.0 per cent on the dry matter basis or 70.4 per cent on a 12 per cent-moisture basis.

TABLE 5
*Digestion coefficients and average chemical composition (7 analyses)
of dehydrated sweet potatoes*

Material determined	Digestion coefficients	Average composition (dry basis)
	(%)	(%)
Crude protein	3.19 ± 3.11	4.86 ± 0.14
Ether extract	52.04 ± 5.74	0.82 ± 0.13
Crude fiber	-51.56 ± 21.61	3.25 ± 0.22
Nitrogen-free extract	90.08 ± 0.43	87.56 ± 0.47
Ash	3.49 ± 0.03

DISCUSSION

The results of this experiment are in line with the results of other work with dairy cows, namely that of Copeland (5) and Rusoff *et al.* (14). In the present trial, when sweet potatoes replaced all of the corn, they were found to be 91.4 per cent as valuable, and when they replaced only half of the corn, they were found to be 94.8 per cent as valuable. On the basis of the calculated total digestible nutrient values, the sweet potatoes were 93.2 per cent as high as corn when compared with the values listed by Schneider (15) for corn grain, found by difference when using cattle for test purposes.

The results on carotene and vitamin A were as expected and corroborate the results of Copeland (5). Dehydrated sweet potatoes have special value for maintaining the carotene and vitamin A in the body and in the milk during the barn feeding period when the quality of the hay is poor, especially when no silage is available.

The standard error per cow and the coefficients of variation for the fat-corrected milk, butterfat test, and body weight were about normal for this type of experiment. In the case of blood plasma carotene and vitamin A, and milk carotene and vitamin A, the coefficients of variation were quite high, but the differences were large enough that the large error terms did not prevent obtaining statistically significant differences.

In the digestion trial the digestibility of the most important constituent, nitrogen-free extract, was high and the results with the four animals were quite uniform. The digestibility of protein was low and not significantly different from zero, but may be quite inaccurate due to the small amount present. The digestibility of crude fiber as determined with each of the four animals was a negative value, but the average was not significantly different from zero. Results on crude fiber at Oklahoma (3) and Louisiana (14) also were negative in some cases.

These results would seem to support the theory that with the increase in the amount of readily soluble carbohydrate in the rumen, the digestibility of the fiber actually may be decreased, through selective use by the rumen organisms. With the small amounts of protein, fat, and fiber present in the sweet potatoes, it might be said that the low digestibility is not important. However, since both the protein and fat already are low compared with corn, the low digestibility merely aggravates a shortage which must be made up by supplying other feeds.

The palatability of the sweet potatoes was quite satisfactory. Three of the cows ate up to 17.6 lb. of sweet potatoes per day in addition to 3.4 lb. of soybean meal. For short periods of time, one of these high-producing cows showed a tendency to leave some of the ration.

The only abnormal results of the experiment were obtained with one of the high-producing cows, whose feces became quite watery. Drug treatment failed to clear up the condition. The appetite was normal and the amount of milk produced continued the same. However, during the period when she was receiving the sweet potatoes, the butterfat test dropped well below normal, averaging 2.4 per cent for four tests. This one cow was largely responsible for the lower fat test for the sweet potato ration. After the discontinuation of the experiment, however, this cow remained in a diarrheic condition for quite some time; this might have been expected as she was turned to pasture after the experiment. In the digestion trial the feces did indicate a slightly more laxative effect for the sweet potato ration than the all-hay ration.

In some areas dehydrated sweet potatoes would seem to show definite possibilities in replacing part of the corn or other carbohydrate feed, if the lower protein and fat are taken into consideration in balancing the rations. At certain times of the year and for certain milk producers, the high carotene content has a special value above the value as a carbohydrate feed.

SUMMARY

1. Dehydrated sweet potatoes when fed to dairy cows in a 75-day double change-over experiment were found to have 91.4 per cent the value of ground yellow corn when they replaced all of the corn. When they replaced only half of the corn, they were 94.8 per cent as valuable.

2. In a digestion trial using four mature dairy cows, the main constit-

uent, nitrogen-free extract, was found to have a digestibility of 90.08 ± 0.43 per cent. On the basis of the digestion coefficients found in this experiment, the total digestible nutrient value was found to be 70.4 per cent on a 12 per cent-moisture basis.

3. Dehydrated sweet potatoes were found to excel corn in maintaining a high level of carotene and vitamin A in the blood plasma and milk. Therefore, they sometimes would have special values which would counteract their slightly lower milk-producing value.

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A SIMPLE COLOR TEST AS AN AID IN GRADING FARM-SEPARATED CREAM^{1, 2}

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It is widely recognized by the creamery industry that a simple test to support the organoleptic method of grading cream would be highly desirable. Although the flavor and odor method is the most satisfactory means available for grading cream for butter making, it has several disadvantages when used by cream station operators as a basis of payment to producers. The chief criticisms are the variability in results due to the personal factor and the lack of visual evidence to support the basis of payment. Supplemental tests for mold, sediment, and acidity often are used for evaluating certain quality factors. However, the correlation between the results of these tests and organoleptic quality is lower than is desired. Furthermore, with the exception of the rapid acidity test, the supplemental tests often are too time consuming for practical use in cream stations for establishing the grade of cream before purchase.

Tests for protein and fat decomposition, even in simplified forms, largely are limited to laboratory use. In addition, their individual relationship with organoleptic grade appears to be too limited for general acceptance as a single measure of quality. No single test would be expected to detect the many possible defects contributing to poor quality. Flavor and odor undoubtedly will remain the principal criteria. Nevertheless, in view of the desirability of a rapid test having high correlation with quality as measured organoleptically and which might be used by cream buyers, field workers and inspectors, data are presented on a method devised for this purpose. The procedure is an outgrowth of observations made during the testing of cream for mold by the Parsons' modification (3) of the Wildman methylene blue-borax method (4). In the latter test it was noted that high quality cream often produced a light colored mixture while poor cream usually produced a darker shade. This observation prompted the testing of various dyes, indicators, and reagents to develop a procedure in which the color obtained with cream would show a suitably close relationship with quality.

MATERIALS AND PROCEDURE

In developing the procedure, the primary objectives were to attain accuracy and simplicity, and to utilize as far as possible facilities and equipment commonly available in cream stations or small plant laboratories. These requisites have governed the quantities of cream and reagents used.

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Crystal violet solution. After preliminary tests with various dyes and indicators, crystal violet was selected as the most suitable for the test. The most satisfactory concentration in water had an optical density of 0.136³ after further dilution of 1:250 to facilitate reading. With the lot of dye⁴ used in this work the required concentration was obtained by dissolving 0.5 g. in 1 l. of distilled water and adjusting by further slight dilution in accordance with optical density readings. Although the solubility of the dye in water at 26° C. is given as 1.68 per cent, some sedimentation occurs in the 0.05 per cent solution after prolonged standing. It should be agitated before use and for obtaining consistent results in photometer readings. The dye solution at the concentration used was reasonably stable at room temperature. When held stoppered in 100-ml. to 500-ml. quantities away from direct sunlight, it remained satisfactory for use over periods up to 3 months.

Sodium hydroxide. Exploratory trials with the separate reagents used in the methylene blue-borax test and with several other bases showed that the color differences obtained with 0.1 *N* sodium hydroxide were the most closely associated with cream quality variations. This reagent accordingly was used in subsequent work. When kept stoppered, the 0.1 *N* sodium hydroxide remained of satisfactory concentration over a 6- to 8-week period. Since weaker base results in darker tests, occasional checking against 0.1 *N* hydrochloric acid is desirable if there is doubt as to the concentration of the sodium hydroxide.

Color standard. A color standard was used to indicate the line of demarcation between first and second grade cream. It was prepared to have the same color value as the majority of tests on cream of borderline quality (between first grade and second grade). Under the conditions of the test, and in accordance with the cream grade standards generally accepted in the Kansas area, this color value was near that commonly termed "Iris". Under a daylight-type fluorescent light it was similar to the color value designated as 43-6B in the "Dictionary of Color" (2). The color was reproduced by experimental mixing of white, red, and blue artists' oil paints. It then was applied to the outside of the lower half of a test tube (outside diameter 15 mm). Several tubes were prepared with slight variations in shade and intensity, so that, when dry, the one most closely representing the desired color under a daylight-type fluorescent lamp could be selected. For protection of the dried paint the tube was inserted into a larger test tube (inside diameter 16 mm.) and held in place with a cotton plug and

³ A Coleman spectrophotometer, model 11, and a 0.5-cm. absorption cell were used. Readings were made at 580 mμ, at which setting the dye exhibited maximum absorption with this instrument.

⁴ Distributed by Coleman and Bell Company, Norwood, Ohio. Cert. No. CC 10. Dye content 89 per cent.

cork stopper. The standard was kept in a pasteboard tube when not in use to minimize the possibility of fading. A 2-oz. sample jar painted on the lower half of the inside also was used occasionally as a standard.

Comparator box. Experimentation showed that comparisons of color value could be carried out best by using a small box, open at the front, as shown in figures 1 and 2. A hole was made in the center of the top side for the color standard tube. The box was made large enough to accommodate a 2-oz. sample jar at each side of the color standard. A blue background intensified differences in shade and gave more satisfactory results than the neutral gray usually recommended for color matching. Color

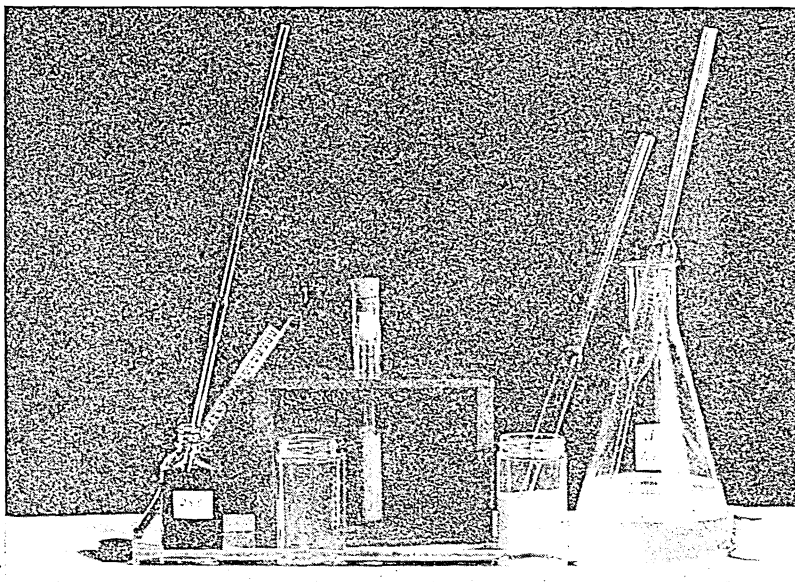


FIG. 1. Materials used for grading cream by the color test.

comparisons on tests were made by placing the box with tests so that light would shine in without shadows.

Light source. The light used for reading the tests affects the color comparison. Apparent changes in color as a result of different types of illumination are more marked in the tests than in the color standard. Since there is considerable variation in the intensity and the color of light in cream stations and other places where cream is graded, it is desirable to have a source of uniform light that will show consistent colors and emphasize small differences. As with color matching generally, a clear north sky light usually is satisfactory. Where this is not available conveniently, a 15- or 20-watt daylight-type fluorescent lamp which gives a slightly bluish light is desirable.

Glassware (fig. 1). The remaining equipment used for making the

test includes the following: 2-oz. cream sample jars (tall type), 1-ml. pipette, 9-ml. pipette, 17.6-ml. pipette, stirring rod, thermometer, bottles for solutions.

Procedure for making the test. Warm sample at about 100° F. until fluid and mix as for Babcock test, pipette 9 ml. of cream into a 2-oz. sample jar, add 17.6 ml. warm 0.1 *N* NaOH (about 120° F.), stir, add 1 ml. crystal violet dye solution and stir. Compare the test sample with color standard in comparator box under daylight-type fluorescent light or clear north sky light. Since the color fades fairly rapidly with cream of good quality, the tests should be read within 1 to 2 minutes after addition of the dye.

Interpretation. As demonstrated later, the color value obtained in the

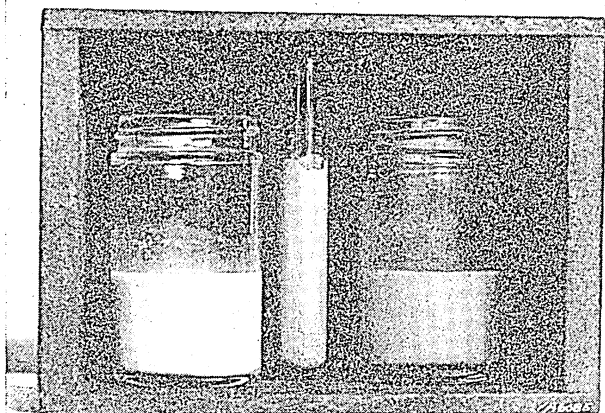


FIG. 2. The color test on first grade cream (left) and second grade cream (right) showing comparisons with color standard. Color differences are more obvious in the actual tests than in the black and white illustration.

test is primarily a function of both the acidity and the physical condition of the cream. It is based on the fact that crystal violet gradually is decolorized at pH values above 10.5 and that the depth of color obtained on the addition of dye to cream is influenced by the dispersion of cream constituents, more homogeneous distribution giving lighter shades. As cream deteriorates, such recognized changes as coagulation of casein, concentration of fat, separation of serum and other probable physico-chemical changes result in a less homogeneous medium than fresh cream and cause a deeper color with added dye. The physical change is augmented by the stirring and agitation the cream receives during accumulation under practical conditions of production.

With few exceptions the shade (lightness or darkness) of the test varies with the quality of the cream, being a light color with good cream and considerably darker with poor cream (fig. 2). Since the color standard

was prepared to have the same value as tests on cream of borderline quality (between first grade and second grade), those tests lighter than the standard are first grade and the tests darker than the standard are second grade or lower. The darkness or lightness of the color also indicates whether the quality corresponds with the upper or lower range of each grade. Tests with the same color as the standard indicate cream of borderline quality. Because an insufficient number of commercial cream samples of reject quality were obtained to establish a color value for this type of cream, such a color standard was not prepared. Even without this standard, experience in using the test would enable the operator to decide on the color value associated with unacceptable cream. When a variety of commercial reject quality cream samples could be obtained for comparative purposes, the necessary color standard could be prepared.

Comparison of color tests with organoleptic grades. The test was used on 780 samples of cream during spring, summer, fall and early winter.

TABLE 1
*Variations between organoleptic grades of commercial
cream judged by individual graders in pairs^a*

Series ^b	Total samples graded	Whole grade variations		Borderline varia- tions resulting in different grades		Total grade variations	
	(no.)	(no.)	(%)	(no.)	(%)	(no.)	(%)
1	85	8	9.4	10	11.8	18	21.2
2	147	16	10.9	8	5.4	24	16.3
3	108	7	6.5	18	16.7	25	23.2
Summary	340	31	9.1	36	10.6	67	19.7

^a Of the two graders for each series, one was the same in all series.

^b Each series represents a different set of samples and a different pair of graders.

Most of the samples represented commercial cream as received at cream stations and creameries. The remainder were experimental samples. The result of the test on each sample was compared with the organoleptic grade. Most of the samples were graded by pairs of graders, with each individual working independently. The remainder of the samples were graded by one grader.

RESULTS

Variation between graders when using organoleptic methods. In order to judge the value of the color test for grading purposes, it seemed desirable to have some information regarding the extent of variation usually prevailing between experienced graders on the same cream when grading by flavor and odor. Table 1 gives the results obtained with three series of samples of commercial cream, with two graders for each series, one of whom was the same in all series.

The results show there was disagreement in grades (including borderline differences) in 16.3 to 23.2 per cent of the cases, or an average of 19.7 per cent. Since, under the conditions involved, the graders undoubtedly graded more carefully than they would have done otherwise, this variation probably is less than would be obtained under practical operating conditions. From general observations it is believed that, with graders who have not worked together previously, agreement on grades of commercial cream probably would not be greater than 80 per cent. Accordingly, it is evident that the organoleptic grade of cream is not an absolute measure of quality. It is not an ideal standard by which to measure a proposed test, since differences may be due to inaccuracies in either method. Nevertheless, the organoleptic method is still the most practical, comprehensive and generally-accepted method of indicating cream quality, and state grade definitions are based largely on such an examination. Therefore, it was used as a standard in evaluating the color test described.

Agreement of color test with organoleptic grade. In the 780 samples of cream graded by both the described color test and by organoleptic means, the two methods agreed in 693 or 88.8 per cent of the cases. This compares with the approximately 80 per cent agreement obtained between human graders. Of the 11.2 per cent of the cases where there was disagreement between the color grade and the organoleptic grade, 77 (9.9 per cent of the total) were borderline differences as between a low first grade and a high second grade. Due to the human factors involved in the organoleptic method, there is some question as to whether these borderline differences really indicate inaccuracy of the color test. In only 1.3 per cent of the samples was there disagreement to the extent of a full grade, whereas experienced graders disagreed by a full grade on an average of 9.1 per cent of the samples graded (table 1).

Of the 780 samples which were graded either as first or second grade, 171 or 21.9 per cent were graded as second grade by the organoleptic method while 159 or 20.4 per cent were graded as second grade by the color test method. The color test placed 12 fewer samples (1.5 per cent) in the second grade than did the organoleptic method. This difference, however, is not significant, as indicated by a chi-square test which gave a value of 0.553 with one degree of freedom.

Consistency of the test. In order to determine if the test gave consistent results under similar conditions, 24 different samples of cream were tested in triplicate and comparisons made of the color of the three tests from each sample. No difference could be detected among the triplicates on any of the samples, indicating that the test gave consistent results when conditions were similar. This supported many general observations made during experimental work.

Agreement in reading of color test by different individuals. In order

to determine whether or not differences between individuals in reading the color test would result in significant differences in cream grading, samples from 30 different lots of cream delivered to cream stations by producers were tested. They then were read independently by three individuals using the same light source. Two of the judges had no previous experience in reading the test. The tests were read as lighter, darker, or the same as the standard, corresponding to first grade, second grade, and borderline quality cream. A chi-square test devised by Friedman (1) for ranked data was used to test the agreement among the three judges on the 30 samples. The chi-square was 1.52 with two degrees of freedom; hence it was concluded that the agreement among these individuals in reading the test was entirely satisfactory. Accordingly, since the procedure involved is simple, it is considered that the test is applicable for cream grading, even by inexperienced individuals.

Factors Determining the Color Value Obtained in the Test

As previously stated, the color value obtained in the test is governed principally by the acidity and physical condition of the cream.

Cream acidity. Although crystal violet is not usually considered to be an indicator, it is decolorized at pH values above 10.5. The change is slow at values between 11 and 12 but is more rapid with increasing alkalinity. Accordingly, in the test as applied to cream, one of the principal factors governing the depth of color obtained is the excess alkalinity after the addition of the NaOH, which is influenced by the acidity of the cream. With cream that is almost sweet (0.2–0.3 per cent titratable acidity), the color of the test is light at the start and fades relatively fast. With high acid cream (either from added lactic acid or natural development) the color is much darker and fading is slower. The color differences between the pale hue obtained with fairly sweet cream and the dark shade obtained with high acid cream correspond to a wide range of alkalinity in the tests and generally represent a titratable acidity range in cream as wide as usually is encountered under practical conditions. Hence the test is a partial measure of cream acidity.

Physical condition of the cream. The influence of the physical condition of the cream was demonstrated by the fact that partially churned cream gave darker color tests than the same cream before agitation. In other trials occasional stirring of cream during holding in the laboratory resulted in darker tests than when no stirring was used, even though the final acidity of the stirred and the unstirred cream was practically the same. The effect of the physical condition was further illustrated by using a laboratory hand homogenizer to redisperse the constituents in old, low quality cream. Such treatment presumably resulted in a more homogeneous medium, similar to fairly fresh, good quality cream. The results of

six trials with sweet cream, sweet cream plus lactic acid, and old, low quality cream are given in table 2.

With sweet cream, homogenization gave no observable difference in the color test after most of the air incorporated had an opportunity to escape. When lactic acid was added to the sweet cream, the color test was darker. Homogenization of the cream with added acid caused only a slightly lighter color. This would be expected where the depth of the color was largely the result of acidity rather than of various physical changes in the cream. With second grade cream, however, the situation was different. Homogenizing the cream produced a much lighter color than was obtained in the test on the unhomogenized cream. This would indicate that the depth of

TABLE 2
Effect of homogenizing the cream (in NaOH) on the color grade

Sample no.	Description of cream	Titratable acidity	Color grade	
			Homogenized ^a	Not homogenized
		(%)		
1	Sweet	0.23	1 + ^b	1 +
2	Sweet	1 +	1 +
3	Sweet + lactic acid	0.70	1	1
4	Sweet + lactic acid	0.79	1 -	1 -
5	Second grade cream	1.10	1 -	2 -
6	Second grade cream	1	2

^a In homogenizing, air is incorporated and influences the color somewhat. Hence color comparisons were made after most of the air had an opportunity to escape.

^b + indicates upper range of grade and - indicates lower range of grade.

color was influenced partly by acidity and partly by physical dispersion of the cream constituents.

Although the color obtained in the test is related independently to both the acidity and physical condition of the cream, the combined effect of these two factors gives results more closely related to organoleptic quality than is produced by either factor alone. The fact that the influence of acidity sometimes is modified by the influence of physical condition and vice versa apparently is the reason for the relationship under practical conditions. Examples are presented in table 3 to show that although the color grade generally is associated with acidity, there are exceptions. These exceptions particularly are evident in samples of high acid cream that were of clean flavor and smooth texture. Such samples were of higher organoleptic quality and showed higher color grades than indicated by titratable acidity. On the other hand, some cream samples of lower acidity were also of low quality as shown by both organoleptic tests and the color tests. Such variations between organoleptic grade and cream acidity generally are recognized, and in this respect the color test is in accord with the organoleptic method.

Many other observations made while using the test indicated that the physical condition of the cream (probably associated with the physico-chemical condition) modified the color obtained in the test. Smooth, clean, high-acid cream often graded higher by the test than did other cream of lower acidity but which was grainy, curdled, partially churned, or had other physical characteristics usually associated with low quality cream. In this characteristic the test agrees with recommended grading practices.

Fat content of cream. Tests on sweet cream of 35 to 40 per cent fat content showed very little difference in color from tests on the same cream diluted to 20 to 25 per cent fat with skim milk. The same dilution with water caused a slightly darker test. Apparently variations in fat content

TABLE 3
Variations between titratable acidity and grade of cream

Sample no.	Titratable acidity	Color grade	Organoleptic grade	Remarks
1	0.20	1 + ^a	1 +
2	0.51	1 +	1 +
3	0.53	1	1
4	0.53	1 -	1 -
5	0.61	2 +	1 -	Thin, watery
6	0.65	1	1
7	0.68	1 +	1	Clean
8	0.73	1 -	1
9	0.73	2	1 -
10	0.91	2 +	2
11	1.08	2 +	2 +
12	1.10	2 -	2
13	1.15	1 -	1 -	Clean, smooth, high acid
14	1.15	2 +	2
15	1.20	1 -	1 -	Clean, smooth, high acid
16	1.24	2	2 -

^a + indicates upper range of grade and - indicates lower range of grade.

within the range commonly encountered under practical conditions have only a minor influence on the color obtained in the test. That the depth of color obtained is not dependent primarily on the fat content also is shown by the fact that lots of the same cream will give different color tests when held under different conditions resulting in quality variations.

Amount of cream in sample. Even with warm fluid cream there is some variation in the amount of cream measured from different samples. Observations where weighed samples were compared with measured samples and where measured amounts were varied by 0.5 ml. showed that such differences in size of the sample had little influence on the color obtained in the test.

Color and thickness of glass in sample jars. Comparisons indicated that differences in the common 2-oz. sample jars did not cause observable differences in the apparent color of the test.

DISCUSSION

It is unlikely that organoleptic grading of cream, where carefully applied, will be satisfactorily replaced by other tests. However, in circumstances where it has been difficult to promote cream grading, where quality is questionable and where such grading is most needed, it is evident that some simple cream grading aid is desirable. It is considered that the test described has merits in this respect. Although the test was studied under Kansas conditions and standardized to the quality standards and grades in that state, it easily could be adapted to conditions existing in most of the Middle-west area where cream stations are common.

With the exception of the color standard, the test utilizes simple, readily available equipment and is rapid and easy to operate. Although laboratory facilities are necessary for their preparation, the reagents are sufficiently stable for use over a period of several months if kept stoppered. Accordingly, if the reagents and equipment were assembled in the form of a field kit, subsequent operation of the test in stations or factories would be simple.

With few exceptions, the test showed a marked difference in color between good and poor cream. In areas and during seasons when the main cause of low cream quality is deterioration, the test should be useful for grading and in visually demonstrating to cream buyers and producers the different quality grades of cream. The fact that the color value obtained in the test, although influenced by acidity, also is modified by the physical condition of the cream is advantageous. Under practical conditions of production and marketing farm-separated cream, there seems to be a close relationship between the age and quality of cream and its physical condition. Presumably this is due partly to the fact that the longer it takes to accumulate, the more stirring or agitation the cream receives. Also, with increase in age and acidity, the physical or physico-chemical condition undoubtedly is modified, as evidenced by easier churning or whipping. Even at fairly low temperatures there is some separation of fat and serum. Although such changes may not always be evident to the eye, they sometimes are emphasized by the tendency of the cream to oil off on the addition of hot water. General recognition of the relationship between quality and physical condition of cream is indicated in the cream grading laws of several states, which stipulate that first grade cream must be smooth and free from lumps, and that lumpy, curdy cream is second grade. The characteristic of the color test of grading down the latter type of cream is in accordance with recommended grading practices.

Where state cream grade definitions place an acidity limit on grades, it would seem that a rapid acid test often would be a necessary complementary test to any other grading method. Although the described color test correlates fairly well with acidity, and the color standard could be modified to compare with desired acidity limits, other factors associated

with quality also are measured by the test. In areas where cream quality generally is high and deterioration is a relatively minor factor compared with other flavor defects (absorbed, weed, feed, etc.), the test would be expected to show less correlation with quality measured organoleptically.

SUMMARY AND CONCLUSIONS

1. A simple color test as an aid in grading farm-separated cream at time of purchase is described. Instructions for the preparation of reagents, description of equipment needed and details for the testing procedure are given.

2. Although the preparation of reagents and the color standard requires laboratory facilities, the operation of the test is simple and is particularly suitable for field work and cream station conditions.

3. The test is based on the depth of color resulting from the addition of 17.6 ml. of 0.1 *N* NaOH and 1 ml. of crystal violet dye solution of given concentration to 9 ml. of cream in a 2-oz. sample jar. Under the conditions of making the test, the color value obtained primarily is related to the acidity and the physical condition of the cream. Under practical conditions of production the combined acidity and physical condition of cream appear to correlate closely with organoleptic grade.

4. Comparison with a prepared color standard of the color value obtained on cream tests permits cream to be graded as first or second grade, and also usually indicates whether it falls in the upper or lower range of the grade.

5. The test was used on 780 samples of experimental and commercial cream from stations and creameries during late spring, summer, fall and early winter. Agreement with organoleptic grades was obtained to the extent of 88.8 per cent of the cases. Most of the differences were only borderline variations. In only 1.3 per cent of the samples did the difference represent a whole grade. This was closer agreement than was obtained between organoleptic grades as judged by experienced graders.

6. It is expected that the test would be most applicable in those areas where poor cream quality is largely the result of deterioration rather than of flavor defects of other types. Since the test is a partial measure of acidity, the color standard could be modified so that the test would be useful even in states where specific acidity limits are placed on cream grades.

7. From the results obtained it appeared that the test offers a means of promoting cream grading in localities where little grading is practiced and where general improvement in quality is needed.

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THE EFFECT OF PREPARATION OF THE COW ON THE RATE OF MILKING^{1, 2}

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One of the important jobs in the management of a dairy herd is that of milking. Several workers have reported on various aspects dealing with this problem. Studies by Zwart (11) and Tgetgel (10) showed a gradual rise in udder pressure from one milking to the next. Gaines (6), Tgetgel (10) and Krzywanek and Brüggemann (8) noted marked increases of intraglandular pressure after mammary stimulation.

Gaines (6, 7), Espe (4) and Foot (5) observed latent periods of varying lengths after stimulation before a "let-down" or excretion of milk occurred.

Elting and LaMaster (2) studied the effect of foremilk on the rate of mechanical milking. They reported that foremilk increased the rate of milk flow in the earlier part of the milking process but prolonged the time required for stripping. The over-all effect was an increase in the total time required for milking. Dodd and Foot (1) found that stimulation before milking shortened the time required for milking. The object of this experiment was to ascertain the effect of preparation or obtaining a let-down of milk before attachment of the milking machine on the rate of milk withdrawal.

EXPERIMENTAL PROCEDURE

Four 2-year-old Holsteins, E401, E405, E413 and A55, and one of mixed breeding, E408, and a 5-year-old grade Holstein, A30, were used in this study. Cows E401 and E405 were hand milked before the beginning of this experiment. Cows E408, E413 and A55 were milked by machine beginning 3 days after calving. A30 was in her third lactation and had been milked by machine in previous lactations.

During the course of the experiment the cows were milked twice daily at 12-hour intervals. At the evening milking of one day and the morning milking of the following day, the cows were stimulated before milking. At the successive evening and morning milking the cows were not stimulated previous to milking. The cows were stimulated to let down or excrete

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milk by a 10- to 15-second wash and massage of the teats and udder with water at a temperature between 120 and 130° F. 2 minutes before the milking process began. Before attaching the teat cups, each quarter was fore-milked by expressing two streams of milk from each teat. For the non-stimulated milkings, the teat cups were merely attached without prior washing or massaging of the teats or udder.

The milking machine was suspended from a scale and readings were taken every 10 seconds from the time the last teat cup was put on until the "end-point" of milking was reached. For the purposes of this study the end-point of a milking was taken as the time after milking when the increment in yield of three successive 10-second scale readings was three-

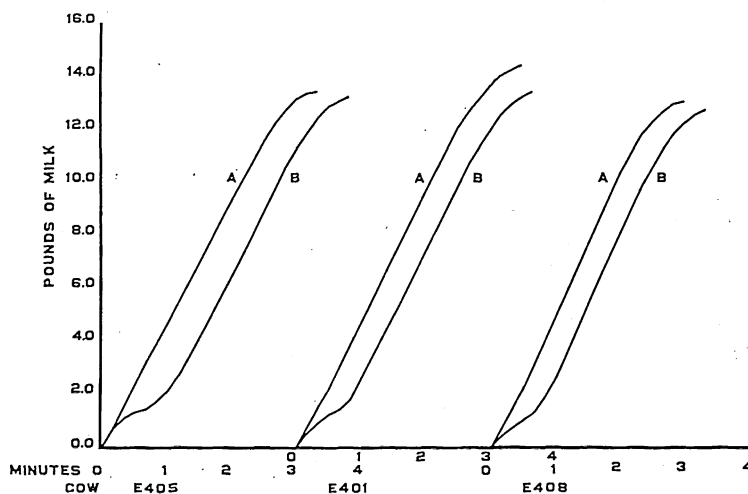


FIG. 1. The effect of stimulation and non-stimulation on the shape of the milk curve. A—Stimulated; B—Not stimulated.

tenths of a pound or less, or when two successive readings were identical. The criterion used depended on which occurred first. The use of an end-point was justified by the fact that preliminary readings had shown that the rate of flow was practically constant to the end-point and machine stripping should occur at that stage of the milking process. A stop watch was used for timing purposes. Ten milkings were recorded for each régime. The milking machine used was a double-action, constant-vacuum type and was operated at 15 inches of mercury negative pressure, commonly called vacuum, on the line and 50 pulsations per minute. There was a difference of 0.5-inch mercury negative pressure between the line and milk hose when milk was not flowing.

RESULTS

Table 1 presents the results of this study. Figure 1 shows the milk curve for three of the cows. A lapse of 30 to 60 seconds occurs before the

TABLE 1
Mean accumulative total pounds of milk for ten milkings when the cows were stimulated and not stimulated before attaching the milking machine

Milking time (minutes and seconds)	Cow E405		Cow E401		Cow E408		Cow A30		Cow A55		Cow E413	
	Stimu- lated	Not stimu- lated	Stimu- lated	Not stimu- lated	Stimu- lated	Not stimu- lated	Stimu- lated	Not stimu- lated	Stimu- lated	Not stimu- lated	Stimu- lated	Not stimu- lated
0: 10	(lb.) 0.71	(lb.) 0.65	(lb.) 0.71	(lb.) 0.48	(lb.) 0.65	(lb.) 0.41	(lb.) 0.98	(lb.) 0.56	(lb.) 0.58	(lb.) 0.32	(lb.) 0.78	(lb.) 0.53
0: 20	1.52	1.08	1.46	0.86	1.39	0.73	1.75	0.77	1.21	0.70	1.55	1.16
0: 30	2.33	1.29	2.21	1.15	2.22	0.98	2.52	0.88	1.85	1.09	2.43	1.66
0: 40	3.18	1.43	3.03	1.42	3.08	1.30	3.38	1.10	2.49	1.47	3.28	2.09
0: 50	3.95	1.65	3.88	1.83	3.99	1.90	4.20	1.62	3.15	2.03	4.16	2.77
1: 00	4.73	2.08	4.71	2.56	4.89	2.64	5.04	2.36	3.84	2.63	5.06	3.55
1: 10	5.51	2.70	5.53	3.36	5.82	3.52	5.89	3.23	4.53	3.28	6.00	4.46
1: 20	6.28	3.43	6.38	4.18	6.75	4.41	6.74	4.12	5.21	3.93	6.89	5.30
1: 30	7.04	4.20	7.21	5.01	7.70	5.36	7.63	5.03	5.88	4.61	7.81	6.19
1: 40	7.86	4.98	7.98	5.84	8.65	6.29	8.48	5.91	6.54	5.23	8.65	7.03
1: 50	8.63	5.79	8.90	6.63	9.52	7.19	9.30	6.78	7.16	5.79	9.46	7.84
2: 00	9.40	6.54	9.68	7.45	10.36	8.10	10.15	7.64	7.70	6.52	10.19	8.68
2: 10	10.18	7.38	10.52	8.27	11.08	9.02	10.94	8.53	8.42	7.17	10.94	9.47
2: 20	10.94	8.14	11.33	9.09	11.75	9.86	11.65	9.43	9.02	7.82	11.67	10.15
2: 30	11.64	8.95	12.08	9.93	12.32	10.64	12.34	10.28	9.62	8.42	12.43	10.87
2: 40	12.27	9.79	12.69	10.74	12.74	11.32	12.93	11.06	10.15	9.07	13.15	11.57
2: 50	12.77	10.57	13.22	11.35	12.99	11.87	13.45	11.77	10.60	9.69	13.77	12.29
3: 00	13.17	11.30	13.69	12.04	13.12	12.31	13.95	12.44	10.97	10.21	14.24	12.97
3: 10	13.44	11.91	14.06	12.60	12.59	14.33	13.02	11.28	10.71	14.53	13.60
3: 20	13.62	12.46	14.33	13.03	12.75	14.69	13.55	11.54	11.16	14.71	14.14
3: 30	12.87	14.50	13.32	14.87	14.02	11.71	11.57	14.51
3: 40	13.10	13.46	14.37	11.89	14.70
3: 50	13.27	14.70	12.13	14.82
4: 00	14.85	12.29
Total milk obtained	14.63	14.76	14.97	15.03	14.53	14.53	16.58	16.16	13.05	13.40	15.67	15.95
Per cent total at end point	93.1	89.9	96.9	89.6	90.3	87.7	89.7	91.9	89.7	91.7	93.9	92.9
Mean rates per min.	4.08	3.48	4.14	3.66	4.38	3.84	4.26	3.72	3.36	3.06	4.44	3.72

milk is let down when the cows are not stimulated, as evidenced by examination of the table and the plateaus in the curves. The curves of the rate of milk removal are markedly different in shape when the cows were stimulated and not stimulated before attaching the milking machine.

All six cows had appreciably higher mean rates of removal of milk when stimulated before milking. The rates of milk flow after stimulation for cows A30, A55, E413, E405, E401 and E408 were 0.71, 0.56, 0.74, 0.68, 0.69 and 0.73 lb. per 10 seconds, respectively, whereas the rates for non-stimulation of the cows in the same order were 0.62, 0.51, 0.62, 0.58, 0.61 and 0.64 lb. per 10 seconds. From 10 to 30 seconds less time was required to reach the end-point when the cows were stimulated, and only with cows A30 and A55 was the percentage of the total at the end-point slightly lower than when not stimulated.

A30 was milked for a period of 15 consecutive days to test the response to continuous non-stimulation. The milking machine was operated under the same conditions as in the previous experiment. The mean total at 1 minute for 29 milkings (readings for one milking were missed when the milk hose dropped off) was 2.38 lb. Reference to table 1 shows that the mean total of ten milkings at one minute was 5.04 lb. for A30 when stimulated before milking. The mean total at 1 minute of the continuous non-stimulation milkings was very similar to that of the mean of the ten milkings, 2.36 lb. of non-stimulation when alternated daily with stimulation before milking.

DISCUSSION

A very definite plateau occurred in the milk curves when the cows were not stimulated, as the milk had not been let down and the sinuses were soon evacuated. The results obtained in this investigation are in agreement with the findings of Gaines (6, 7), Foot (5) and Espe (4). Practically a constant rate of flow was obtained from the time the milking process began until the end-point was reached when the cows were prepared for milking by prior stimulation. The initial flow of milk of about a pound when the cows were not stimulated represents the milk that had drained into the large ducts and the gland and teat sinuses.

Ely and Petersen (3) noted a response by the let-down or excretion of milk 45 seconds after the injection of oxytocin. Thus, the plateau in the milk flow curve of cows not prepared for milking by stimulation is the time necessary for the milking stimulus to motivate the posterior lobe of the pituitary to secrete the oxytocic principle into the blood stream and cause a let-down or excretion of milk.

Before stimulation, the teats are soft and flabby, but they become firm and turgid after stimulation as a result of the let-down or excretion of milk, with a resulting increase in intraglandular pressure. It was observed that teat cups are much more easily attached to a turgid than a flabby teat.

Petersen (9) reported that the teat cups crawl or draw in the slackened udder tissue when the intraglandular pressure is low and occlude the orifices between the gland and teat sinuses. The results of this study show that the time required for milking was longer when the cows were not stimulated. Obviously a milking machine attached to the teats of an udder in which an increment of intraglandular pressure has not been effected by stimulation may occlude the passage between the gland and teat at the beginning of milking, thereby prolonging the milking process. In addition, when the sinuses have been drained, as represented by the plateau in the milking curves, the teat cups draw in the flaccid udder tissue and trauma may result to the secretory tissue at the juncture of the teat and gland.

SUMMARY AND CONCLUSIONS

Preparing the cow for milking by stimulation with a wash and massage of the udder with water at 120 to 130° F. was found to increase the rate of milking and decrease the time required for the milking process as compared with no preparation.

A grant from Babson Brothers Milker Company, Chicago, Illinois, that made this study possible is gratefully acknowledged.

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THE REDUCING CAPACITY OF MILK AND MILK PRODUCTS AS MEASURED BY A MODIFIED FERRICYANIDE METHOD^{1,2}

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In 1945 Chapman and McFarlane (5) published a method for determining reducing substances in milk and milk products by heating with potassium ferricyanide under specific conditions. The method was adapted from the procedure used by Anson (1, 2), Mirsky (12) and Mirsky and Anson (13) for determining sulfhydryl groups in proteins. Chapman and McFarlane found that fresh milk possesses considerable capacity to reduce ferricyanide under the conditions used and that heat treatment of milk or storage of milk powder open to the atmosphere increases the reducing capacity.

In 1945 Harland and Ashworth (8) reported the use of thiamin disulfide for estimation of the reducing power of milk. This reagent evidently is a much weaker oxidant than ferricyanide, at least under the conditions used, since it is not reduced at all by normal unheated milk. Heat treatment, however, does produce materials which reduce thiamin disulfide but long continued heat treatment in the presence of air causes a subsequent decrease in reducing power. The disparity in behavior of milk to these two reagents prompted Chapman and McFarlane (6) to express the opinion that these reagents react with different reducing systems. This opinion is somewhat substantiated by the work of Lea (10), which indicates that materials produced by interaction of lactose and protein are responsible for the increase in reducing capacity of dry milk during storage at 47° C. and 55 per cent relative humidity.

The work reported in this paper was undertaken prior to publication of Lea's results to examine the ferricyanide method and to determine which constituents of milk reduce this reagent and contribute to the increases produced by processing and storage.

METHOD

Factors affecting reduction of ferricyanide. The extent to which ferricyanide is reduced by a system such as milk is strongly influenced by the

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hydrogen ion concentration, which determines the reduction potential of the several reductants present. Furthermore, since the reaction is slow, it usually is not allowed to go to completion. Hence the temperature and time of the reaction become of great importance in determining the extent of reduction. Chapman and McFarlane (5) made some study of the effect of the three variables—pH, temperature, and time—on the amount of ferricyanide reduced by milk powder. They showed: (a) that the capacity to reduce ferricyanide increases markedly from pH 2 to pH 8, (b) that the reaction proceeds much faster at 70° C. than at 50° C. (at pH 5.0),

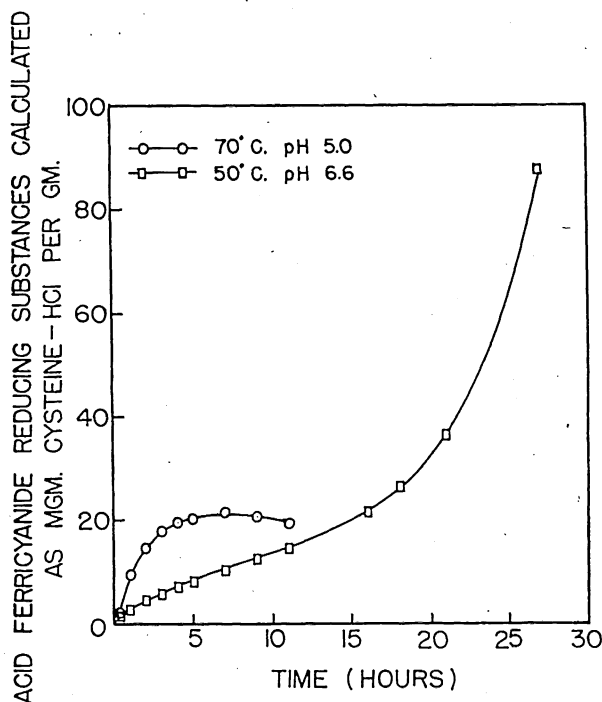


FIG. 1. Reduction of ferricyanide by dry whole milk.

and (c) that the reaction apparently is far from complete in 40 minutes (at 70° C. and pH 5.0). Others (13) have shown that protein continues to reduce ferricyanide for 12 to 24 hours at least. On the basis of their study of the factors influencing the reduction, Chapman and McFarlane (5) adopted the standard conditions of pH 5.0, 70° C., and 20 minutes as giving a satisfactory differentiation between fresh and aged samples.

In employing the method of Chapman and McFarlane, the present authors soon encountered a serious difficulty. In some cases, particularly with powders of high reducing capacity, a blue precipitate was retained on the filter paper when the reaction mixture was deproteinized. This phenomenon could be attributed to partial decomposition of the ferricyan-

ide during heating with liberation of ferric ions which react with ferrocyanide to form Prussian blue (ferric ferrocyanide). Since the extent of reduction is determined by formation of Prussian blue after deproteinization, any such removal of ferrocyanide by "preformation" of Prussian blue might seriously vitiate the results. Consequently, conditions which would obviate this difficulty were sought for conducting the reaction.

It soon was found that by raising the pH to 6.6 and lowering the temperature to 50° C., no Prussian blue was preformed, although the rate of

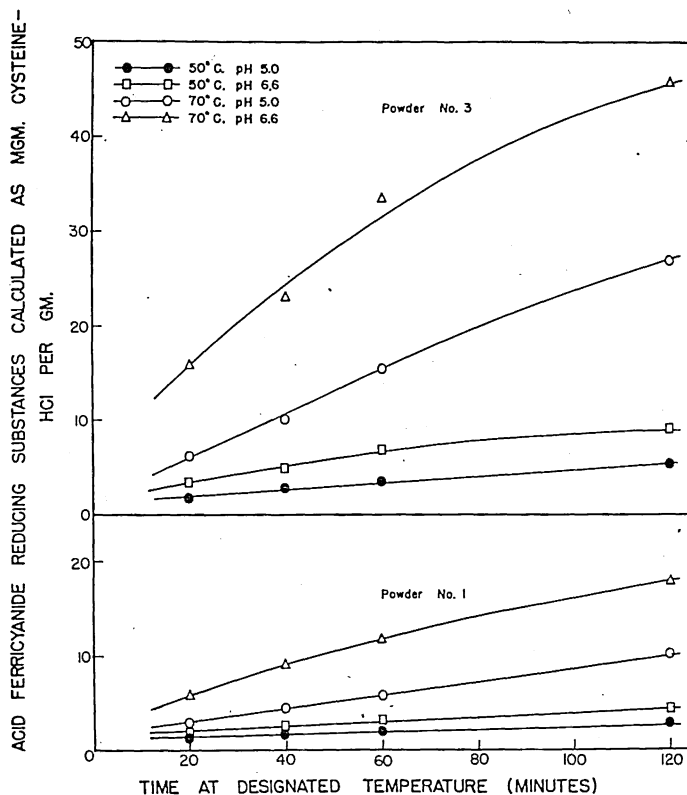


FIG. 2. Effect of temperature and pH on rate of reduction of ferri-cyanide by two samples of dry whole milk.

reduction was somewhat lower than that at pH 5.0 and 70° C. Figure 1 shows a comparison of the rate of reduction at pH 5.0, 70° C., with that at pH 6.6, 50° C. The fact that the reduction at pH 5.0, 70° C. reaches a maximum and even tends to drop off is attributable to the "preformation" of Prussian blue. Evidently, reduction continues indefinitely at pH 6.6, 50° C. Figure 2 shows the rates of reduction of two powders under the following conditions: pH 5.0, 70° C.; pH 5.0, 50° C.; pH 6.6, 70° C.; and pH 6.6, 50° C. Of these four, the last yielded satisfactory differentiation

without the use of excessively high temperature; consequently, it was adopted, together with a standard reaction time of 20 minutes.

Chapman and McFarlane (5) stated that the use of 5 ml. of 1 per cent ferricyanide per 100 mg. of milk powder yielded maximum color intensity. In the present study limited data indicate that greater intensities are obtained by increasing the concentration of ferricyanide. However, this point has not been investigated very extensively, and 5 ml. of 1 per cent solution have been employed routinely.

Folin (7) and Anson (1) have indicated that impurities may be encountered in ferricyanide and have suggested methods for purification. Furthermore, Anson (1) advised storage of ferricyanide at 5° C. in the dark and checking it occasionally for the presence of ferrocyanide. In the present study no evidence of impurities in reagent grade ferricyanide was observed, but solutions of it did deteriorate at room temperature. No evidence of deterioration of solutions stored in the dark at 5° C. for periods up to 15 days has been found.

Factors affecting color intensity. Chapman and McFarlane (5) stipulated the use of "freshly prepared" ferric chloride solution for formation of Prussian blue in the deproteinized filtrate. In the present study, holding such solutions for periods up to 4 days was practically without effect on color intensity, but the general rule of preparing fresh solution each day was adopted.

The procedure of Chapman and McFarlane (5) in reading the color intensity at exactly 10 minutes after addition of the ferric chloride solution was followed. Under these conditions, of course, the color intensity does not follow Beer's law and a calibration curve must be used. Lea (10) has reported that, if the holding period is limited to one minute, Beer's law is obeyed, but in the opinion of the present workers any advantage gained by such a procedure is offset by the fact that slight variations in holding time would introduce larger errors than in the case of the 10-minute holding period.

The method adopted. Weigh a 100-mg. sample of dry milk or simplified system into a test tube (22 × 150 mm.) and disperse it in 5 ml. of distilled water at 50° C. Alternatively reconstitute 5 g. in 250 ml. of distilled water and use a 5-ml. aliquot. Add 5 ml. of a buffer at pH 6.6 (M/5 potassium dihydrogen phosphate and M/5 sodium hydroxide) and 5 ml. of 1.0 per cent potassium ferricyanide. Mix well and heat for exactly 20 minutes in a continuously agitated water bath maintained at 50° C. Cool immediately to 25° C. or lower in an ice water bath. Add 5 ml. of 10 per cent solution of trichloroacetic acid, mix and filter through no. 40 Whatman filter paper. Transfer 5 ml. of the filtrate to a test tube (22 × 150 mm.) and dilute with 5 ml. of distilled water. Add 1 ml. of fresh 0.1 per cent ferric chloride solution and mix thoroughly by vigorous shaking. If several de-

terminations are being made, add the ferric chloride solution to the tubes at intervals of 1 minute and hold each tube for exactly 10 minutes before determining the color intensity. A pair of matched square cuvettes and a Coleman Universal Spectrophotometer have been used, making all readings at $660\text{ m}\mu$ with the reagent blank set to read 100 per cent transmission. The reagent blank is similar to the unknown except that 5 ml. of water is substituted for the sample.

In some cases it was desired to determine the proportion of the reducing capacity contributed by components of the system other than protein. For this purpose a portion of reconstituted sample was deproteinized with

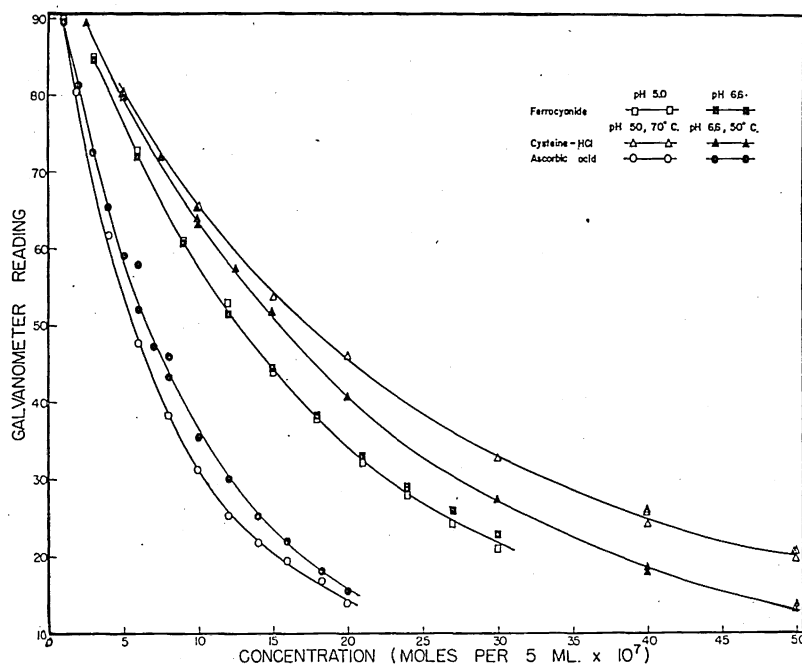


FIG. 3. Calibration curves showing the relation of the intensity of Prussian blue color to concentration of ferrocyanide, cysteine, and ascorbic acid.

tungstic acid and ferrieyanide reduction determined in the filtrate. The use of a buffer having a pH of 7.4 was found necessary to insure a final pH of 6.6. The detailed procedure is as follows: Reconstitute 1 g. of powder with 25 ml. of distilled water in a 50-ml. volumetric flask. Add 16 ml. of 0.33 *N* sulfuric acid, 8 ml. of 10 per cent sodium tungstate and sufficient distilled water to bring to volume. Mix thoroughly, hold for 10 minutes and filter. For the ferrieyanide reduction use 5 ml. of filtrate (equivalent to 100 mg. of powder) and 5 ml. of buffer at pH 7.4. Proceed from this point exactly as for whole milk except that filtration after adding trichloroacetic acid may be omitted.

Expression and reproducibility of results. The choice of units for expression of the reducing power of milk is complicated by the multiplicity of reductants involved. Faced with this situation, Chapman and McFarlane (5) chose to calibrate the method with glutathione and to express the capacity of milk to reduce ferricyanide in terms of the molar concentration of glutathione sulfhydryl groups required for an equivalent reduction. However, Lea (10) contends, with considerable justification, that, in view of the lack of knowledge of the specific groups involved, it is preferable to express reducing power of milk in terms of moles of ferricyanide reduced.

Figure 3 shows a curve relating galvanometer reading to concentration of potassium ferrocyanide. In obtaining the data for this curve a series of solutions containing 1.0 per cent ferricyanide and concentrations of ferrocyanide up to 60×10^{-5} molar was prepared. Five milliliters of such solution, 5 ml. of buffer (either pH 5.0 or pH 6.6), 5 ml. of water, and 5 ml. of 10 per cent trichloroacetic acid then were mixed and a 5-ml. aliquot taken. To this was added 5 ml. of water and 1 ml. of 0.1 per cent ferric chloride and the color intensity read after 10 minutes. Little if any effect of pH on color development was found. Figure 3 also shows curves relating concentration of cysteine and ascorbic acid to the intensity of blue color obtained from ferrocyanide produced by reduction of ferricyanide by these reductants.

In figure 4, the concentration of ferrocyanide necessary to produce a given color intensity has been plotted against the concentration of cysteine or ascorbic acid which produces an identical intensity by reduction of ferricyanide. Ascorbic acid reacts very nearly stoichiometrically at pH 6.6, 50° C. with ferricyanide; the slope of the line indicates that 1 mole of ascorbic acid reduces 1.95 moles of ferricyanide, which is very close to the theoretical equivalent of 2.00. At pH 5.0, 70° C., slightly more than 2 moles of ferricyanide are reduced by a mole of ascorbic acid. Tauber and Kleiner (14) employed a somewhat similar method for determining ascorbic acid but the reduction was carried out at a lower temperature (40° C.) in a more acid medium (10 per cent trichloroacetic). Their data give no evidence as to the stoichiometry of the reaction. (See also Ball (3).)

The extent of reduction by cysteine is neither so complete nor so uniform over the concentration range studied as is that by ascorbic acid. In the range of concentration from 5 to 32×10^{-7} moles per determination, a mole of cysteine reduced about 0.85 mole of ferricyanide at pH 6.6, 50° C., and about 0.70 mole at pH 5.0, 70° C. Mason (11) estimated glutathione by oxidation with ferricyanide at pH 5.9 at room temperature and determination of Prussian blue. The oxidations of cysteine and glutathione were stoichiometrically equivalent under these conditions, but the paper gives no information as to whether the ferricyanide reduced is stoichiometrically equivalent to the cysteine or glutathione oxidized. However,

Anson (1) obtained 0.00104 mM of ferrocyanide when 0.001 mM of cysteine was oxidized with 0.2 mM of ferricyanide for 20 minutes at pH 6.8, 50° C., which does indicate a stoichiometric relation. While these data support Lea's (10) contention that the reduction of ferricyanide by cysteine is not stoichiometric under the conditions used, they do furnish an empirical relation which could be used in converting from one basis to another.

All results are expressed in terms of equivalent cysteine hydrochloride

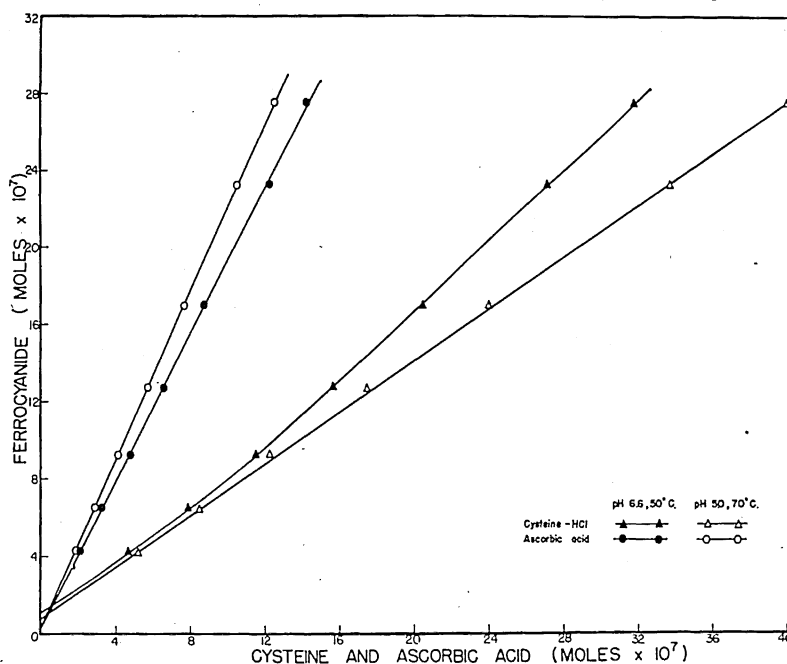


FIG. 4. Relation between cysteine or ascorbic acid oxidized and ferrocyanide produced.

concentrations because such standardization furnishes a common basis for comparing various methods.

To test the reproducibility of results, several 100-mg. samples of two different milk powders were weighed into test tubes which were stoppered and held at -10° C. On several occasions determinations were made on these samples by two individuals working independently. The results, shown in table 1, indicate a rather satisfactory degree of reproducibility for either individual. However, there is a small but consistent unexplainable difference between individuals.

MATERIALS

The materials used in this study were those described in a recent paper on fluorescence (9). Briefly, they were spray dried milk, acid-precipitated

TABLE 1
Comparison of results obtained by two individuals with the ferricyanide method

Trials ^a	Reducing substances as cysteine-HCl per g.			
	Sample 358		Sample 49	
	A ^b	B ^b	A ^b	B ^b
	(mg.)	(mg.)	(mg.)	(mg.)
1	1.32	1.42	2.88	2.93
2	1.32	2.84
3	1.36	2.95
4	1.38	1.41	2.84	3.01
5	1.37	1.45	2.84	2.92
6	1.32	1.41	2.90	2.87
Mean	1.35	1.42	2.88	2.93

^a Each trial made on a different day.

^b Individuals designated A and B.

casein, dialyzed milk serum protein, filtered milk fat, a concentrate of fat globule "membrane" from washed cream, and commercial samples of lactose, riboflavin and ascorbic acid.

RESULTS

Effect of processing on the reducing capacity of whole milk. Chapman and McFarlane (5) have shown that an increase in the temperature of preheating the fluid milk increases the acid ferricyanide reducing substances in spray-dried whole milk. The data presented in table 2 indicate that the reducing capacity of the dry whole milk also is influenced by the temperature of spray drying. The use of higher drying temperatures may, in fact, overshadow the effect of variation in preheating temperature.

TABLE 2
Effect of preheating and spray-drying on acid ferricyanide reducing substances in dry whole milk

Series	Preheat treatment		Reducing substances as cysteine-HCl per g. of solids					
	Temp.	Time	Fresh	Pre-heated	Con-densed	Frozen dried	Spray-dried	
							N ^a	H ^b
	(°C.)	(Min.)	(mg.)	(mg.)	(mg.)	(mg.)	(mg.)	(mg.)
1	66	30	1.77	2.55
2	66	30	1.00	0.96	0.96	1.20	1.88
3	74	30	0.96	1.00	1.08	1.11	1.24	1.58
4	74	30	1.05	1.08	1.18	1.90
5	74	30	0.86	0.86
6	74	30	1.22	1.63
1	85	20	1.80	2.47
2	85	20	1.00	1.14	1.09	1.30	1.97
4	85	30	1.05	1.21
5	85	30	0.86	1.02

^a N = Normal drying temperature.

^b H = High drying temperature.

As is shown by data in table 2, drying from the frozen state under vacuum is essentially without effect on the reducing capacity of whole milk. This fact has been confirmed in experiments with other samples.

Contribution of the constituents of milk to the reducing effect. (a) Caseinate and caseinate-lactose systems—Casein was dispersed in sufficient lime water to produce a sol at pH 6.6 containing 1.0 g. of casein per 16 ml. of sol. Lactose was added to portions of this sol to produce sols with 0, 0.025, 0.05, 0.10, 0.50, 1.0, 2.15 and 4.30 parts of lactose (weighed as α -hydrate) per part of casein. The effect of heat treatment at various

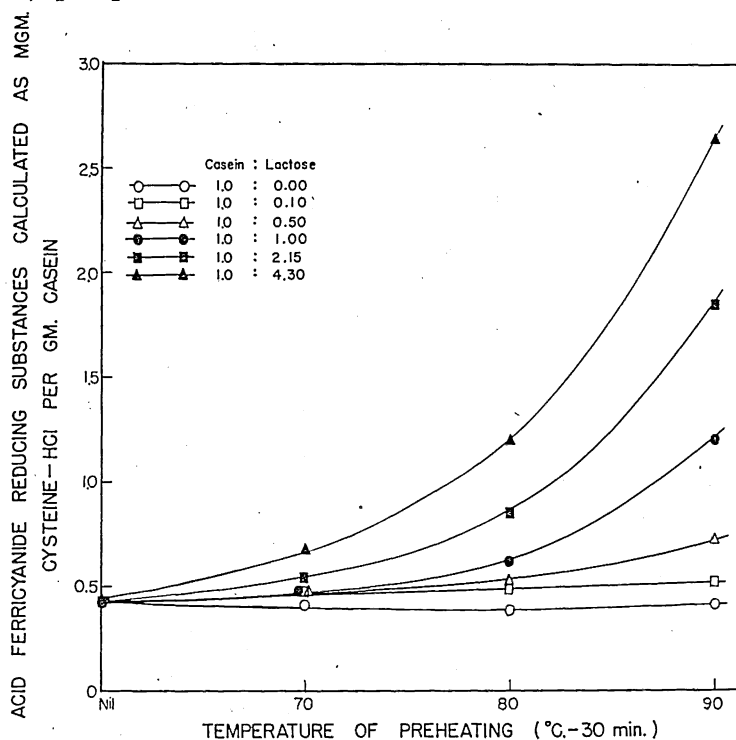


FIG. 5. Effect of heat treatment on acid ferricyanide reducing materials in casein-lactose systems.

temperatures on the acid ferricyanide reducing capacity of these sols is shown in figure 5.

Heat treatment produced no change in the reducing effect of the calcium caseinate sol, but in those sols containing lactose as well as calcium caseinate, there was an increase with increase in temperature, which, at any given temperature, was roughly proportional to the lactose content.

(b) Serum protein and serum-protein-lactose systems—Milk serum protein prepared as described by Jenness and Coulter (9) was equilibrated against phosphate buffer (pH = 6.6, μ = 0.1) and adjusted to a protein con-

centration of 1.0 g. per 100 ml. Lactose was added to give mixtures containing, respectively, 0, 0.164, 1.632 and 7.06 g. of lactose (weighed as α -hydrate) per g. of serum protein. A solution containing 7.06 g. of lactose (α -hydrate) per 100 ml. of the buffer but no protein was included for comparison. The effect of heat treatment at various temperatures on the acid ferricyanide reducing capacity of these systems is shown in figure 6.

In contrast to the effect of heat on the calcium caseinate sol, heating of the serum protein sol resulted in an increase in the acid ferricyanide re-

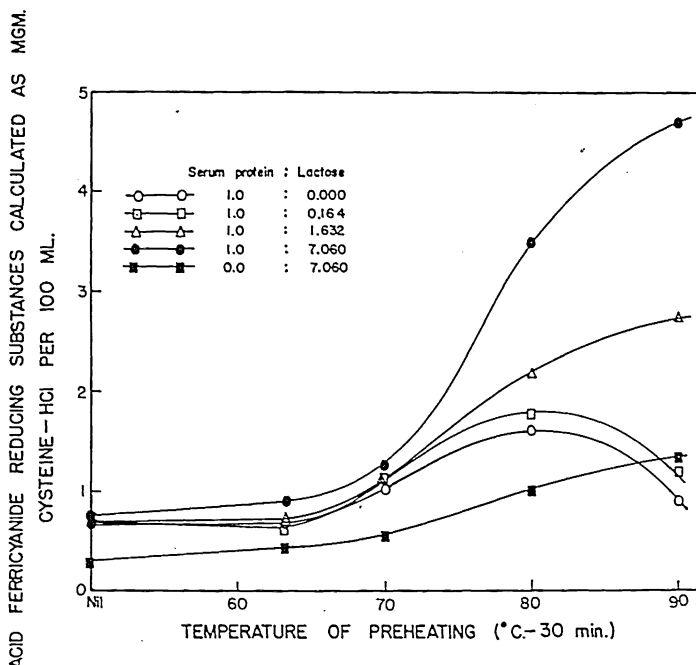


FIG. 6. Effect of heat treatment on acid ferricyanide reducing materials in milk-serum-protein-lactose systems.

ducing effect, but the maximum capacity was not produced by the most drastic heat treatment used. Evidently greater oxidation of reducing groups liberated from the protein occurred on heating at 90° C. Heat treatment of lactose solutions in phosphate likewise produced materials capable of reducing acid ferricyanide. The effect of heat treatment on systems containing both serum protein and lactose was to produce reducing capacities much greater than the sum of those produced from the two constituents separately. These increases were related both to temperature and to the lactose content of the systems.

(c) Systems of calcium phosphocaseinate and other milk constituents dried from the frozen state—As already shown, the drying of whole milk

from the frozen state does not increase its reducing capacity appreciably. The effect of preheating various liquid-simplified systems at temperatures of 74 and of 90° C. for 30 minutes on their reducing capacity after drying from the frozen state is shown in table 3. These systems were prepared as described in a previous paper (9). All of the results have been calculated to the basis of cysteine hydrochloride equivalent per gram of the most complete system (*i.e.*, that containing all of the constituents). Expression of the results in this manner makes immediately apparent the contribution of each constituent to the reducing capacity of the complete system.

The addition of lactose to a caseinate system enhanced both the initial

TABLE 3
Production of acid ferricyanide reducing substances in simplified systems dried from the frozen state under vacuum

System	Constituents ^a	Reducing substances as cysteine-HCl per g. complete system ^b		
		Heat treatment for 30 min.		
		None	74° C.	90° C.
		(mg.)	(mg.)	(mg.)
1	Caseinate ^c	0.05	0.05
2	1 + lactose	0.14	0.20	0.30
3	2 + serum protein	0.20	0.28	0.35
4	2 + milk fat	0.20
5	4 + f.g.m. ^d	0.20	0.25	0.38
6	5 + serum protein	0.36	0.39	0.56
7	6 + riboflavin	0.33
8	7 + ascorbic	0.50

^a Ratio of constituents was as follows: 1.00 casein: 2.04 lactose: 0.30 serum protein: 1.52 milk fat: 0.04 f.g.m.^d: 0.000075 riboflavin: 0.0010 ascorbic acid.

^b All systems dried from the frozen state under vacuum.

^c Calcium phospho-caseinate.

^d Fat globule "membrane".

reduction capacity and the effect of heat, thus confirming the data of figure 5. Serum protein also contributed significantly to reducing capacity and to heat susceptibility, and the materials of the fat globule "membrane" made a small contribution also.

Production of reducing substances during storage. The formation of acid-ferricyanide reducing substances during storage of simplified systems dried from the frozen state was studied. Samples of each system were stored under nitrogen over 45 or 60 per cent sulfuric acid at 37 or 50° C. for varying periods of time. Samples of spray dried whole milk and of whole milk dried from the frozen state were included for comparison. The 45 and 60 per cent sulfuric acid solutions furnished vapor pressures at 37° C. comparable to those that had been found to be in equilibrium with dry whole milk containing 5.4 and 2.7 per cent moisture,

TABLE 4
Reducing capacity of frozen-dried simplified systems and effect of storage thereon

System no.	Constituents ^a	Acid ferriyanide reducing substances as cysteine-HCl per g. complete system									
		Fresh			90 days at 37° C.			30 days at 50° C.			60 days at 50° C.
					Over 45% H ₂ SO ₄			Over 60% H ₂ SO ₄			
		A ^b	B	C	A	B	C	A	B	C ^c	
		(mg.)	(mg.)	(mg.)	(mg.)	(mg.)	(mg.)	(mg.)	(mg.)	(mg.)	(mg.)
1	Caseinate	0.05	0.08
2	1 + lactose	0.15	0.17	0.20	4.95	4.90	0.41	1.11	0.63	1.90	1.46
3	2 + serum prot.	0.22	0.28	0.28	6.47	6.03	0.72	1.90	0.99	2.72	3.40
4	2 + milk fat	0.20	0.21	0.20	5.58	4.98	0.60	1.10	0.91	1.45	2.13
5	4 + f.g.m. ^d	0.19	0.25	0.25	5.50	4.84	0.77	1.60	0.84	1.77	2.07
6	5 + serum prot.	0.32	0.38	0.39	7.46	5.88	0.97	2.41	1.24	1.72	3.09
7	6 + riboflavin	0.22	0.35	0.33	7.85	5.86	0.92	2.25	1.26	1.99	3.06
8	7 + ascorbic	0.49	0.53	0.50	8.16	5.97	1.08	2.05	1.62	2.46	2.99
9	Whole milk:		1.22								
	Frozen dried		1.63		10.28		2.05		3.00
10	Spray dried				8.81		2.33		3.07

^a Ratio of constituents was as follows: 1.00 casein: 2.15 lactose hydrate: 0.30 serum protein: 1.52 milk fat: 0.04 f.g.m.^d: 0.000075 riboflavin: 0.0010 ascorbic acid.

^b Letters designate replicate series.

^c Stored 34 days.

^d Fat globule "membrane".

respectively. The quantities of simplified systems prepared, particularly those containing serum protein, were insufficient to permit satisfactory moisture determinations by the toluene distillation method.

Here again the results have been calculated to the basis of cysteine hydrochloride equivalent per gram of complete system. The data, recorded in table 4 show that even the most complete system employed failed to exhibit a reducing capacity of over half of that of dry whole milk. There was only a slight increase in reducing capacity of the caseinate system during storage. Interaction of caseinate and lactose was responsible for the major portion of the increase produced in storage. Serum protein also contributed materially to the original- and storage-produced reducing

TABLE 5
*Non-protein reducing capacity of frozen-dried simplified systems
and effect of storage thereon*

System no.	Constituents	Acid ferricyanide reducing substances as cysteine-HCl per g. complete system							
		Fresh		90 days at 37° C.				30 days at 50° C.	
				Over 45% H ₂ SO ₄		Over 60% H ₂ SO ₄		Over 60% H ₂ SO ₄	
		A	B	A	B	A	B	A	B
2	Caseinate + lactose	(mg.)	(mg.)	(mg.)	(mg.)	(mg.)	(mg.)	(mg.)	(mg.)
3	2 + serum prot.	0.03	0.05	0.65	0.45	0.12	0.14	0.13	0.10
4	2 + milk fat	0.05	0.09	1.00	0.62	0.22	0.17	0.20	0.22
5	4 + f.g.m.	0.06	0.04	0.68	0.37	0.27	0.15	0.08	0.16
6	5 + serum prot.	0.07	0.09	0.53	0.32	0.12	0.15	0.15	0.21
7	6 + riboflavin	0.08	0.08	0.65	0.41	0.20	0.13	0.35	0.16
8	7 + ascorbic	0.08	0.06	0.85	0.41	0.27	0.16	0.35	0.22
		0.35	0.21	0.94	0.57	0.35	0.19	0.50	0.33
9	Whole milk:								
10	Frozen-dried	0.71		2.41		0.77		0.99	
	Spray-dried	0.68		1.70		0.77		1.04	

capacities. The effect of the fat globule "membrane" materials was small and variable. Milk fat and riboflavin were inert but, as expected, ascorbic acid definitely enhanced the reducing capacity.

The data in table 5 indicate that ascorbic acid is the major non-protein reductant of acid ferricyanide in the fresh systems, but that non-protein reducing materials are produced upon storage by reactions involving the proteins or lactose or both. As was the case with total reducing capacity, the most complete simplified system failed to account for all of the non-protein reducing capacity of dry whole milk.

DISCUSSION

The modified ferricyanide method in which the pH is raised to 6.6 and the temperature lowered to 50° C. appears to yield as satisfactory re-

sults as that originally described by Chapman and McFarlane. It has the advantage of eliminating formation of a blue precipitate during the heating.

Ascorbic acid has been found to reduce ferricyanide stoichiometrically under the conditions adopted for the reaction. The amount of ascorbic acid present in milk, however, accounts for only a fraction of the ferricyanide-reducing capacity. Thus, for a milk containing 20 mg. of reduced ascorbic acid per l. (125 g. of solids), it may be calculated from the standardization curves that ascorbic acid would account for a reducing capacity equivalent to 0.36 mg. cysteine hydrochloride per g. of solids out of a total of about 1.00 mg. per g. Actually the effect of addition of ascorbic acid in the amount of 25 mg. per liter to artificial systems was somewhat less than this, amounting to the equivalent of 0.27, 0.18 and 0.17 mg. cysteine hydrochloride per g. in the three series reported in table 4. Obviously, considerable differences in the acid ferricyanide reducing capacity of milk may result from variation in the degree of oxidation of its ascorbic acid content.

Such relatively simple sulfhydryl compounds as cysteine and glutathione are also effective reductants of ferricyanide under the conditions used. According to Brand and Kassell (4) the cysteine content of β -lactoglobulin is about 1.10 per cent (analyzed after acid hydrolysis). If this figure be assumed to apply to the entire 0.70 per cent serum protein of milk, and if the sulfhydryl groups of milk proteins were as reactive as those of cysteine or glutathione, the protein sulfhydryls of milk would furnish a reducing capacity equivalent to about 0.80 mg. cysteine hydrochloride per g. of solids. Actually, the fact that the contribution of milk serum protein falls far short of this value (see table 4) constitutes evidence that the sulfhydryls of protein are much less active than those of the simpler compounds.

The failure of the most complete simplified system prepared to exhibit more than one-half the reducing capacity of fresh or frozen-dried whole milk could conceivably be due to absence of some milk reducing system from the simplified preparation. On the other hand, the reducing capacity of one or more of the constituents of the simplified system might possibly have been altered in isolation and purification. Such an effect would be most probable with the serum protein. A third possibility is that the environment of the reducing materials in the simplified systems is different enough from that in milk to account for the difference. The results reported in this paper give no clue to the reason for the discrepancy.

In spite of the failure quantitatively to duplicate the reducing capacity of fresh whole milk, the simplified systems do exhibit increases in reducing capacity upon heat treatment and storage which are quite comparable to those observed with milk itself. Furthermore, they indicate that these in-

creases are due to materials formed in part by reactions of the proteins with lactose and in part by reactions involving lactose in the presence of buffer salts.

The method is being applied to further study of factors influencing the changes occurring in dry whole milk during storage.

SUMMARY AND CONCLUSIONS

A modification of Chapman and McFarlane's ferricyanide procedure for evaluating the reducing capacity of milk is presented. This modification, which involves raising the pH to 6.6 and lowering the temperature to 50° C., proved somewhat more satisfactory, particularly with milk powders of high reducing capacity, than the original method. The method has been calibrated in terms both of ferricyanide reduced and of cysteine or ascorbic acid oxidized.

The capacity of milk to reduce ferricyanide is increased both by heat treatment and by spray drying. Study of simplified systems of milk constituents has shown that some of the reducing substances produced by heat treatment of milk and aging of dry milk are formed from lactose and from protein-lactose interactions.

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THE INFLUENCE OF *MYCOTORULA LIPOLYTICA* LIPASE UPON THE RIPENING OF BLUE CHEESE MADE FROM PASTEURIZED HOMOGENIZED MILK¹

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The present trend in the dairy industry is to manufacture cheese from pasteurized milk, both for public health reasons and to aid in the control of microbial defects. When blue cheese is made from pasteurized milk the product does not develop a full and typical flavor during ripening (6, 10); this has been attributed primarily to the inactivation of milk lipase by pasteurization (10), resulting in less hydrolysis of the butterfat of the cheese. The solution of this problem seems to lie in the substitution of a suitable lipolytic enzyme for the milk lipase inactivated by pasteurization. The present study was undertaken to explore the possibility of substituting the cell-free lipase produced by *Mycotorula lipolytica* (15, 16) for normal milk lipase in the manufacture of blue cheese from pasteurized homogenized milk.

HISTORICAL

Methods for the manufacture of blue cheese from raw cows' milk have been described by different workers (6, 12, 18). Lane and Hammer (9) modified the procedure formerly used by homogenizing the raw milk. This modification resulted in faster ripening of the cheese as well as in more luxurious mold growth, as compared with similar cheese made from nonhomogenized milk. Later the same workers (10) reported that blue cheese made from pasteurized homogenized milk was a more satisfactory product than that made from raw, nonhomogenized milk, but less satisfactory than if raw homogenized milk was used. They also observed that milk lipase definitely aided in the ripening of blue cheese. Fabricius and Nielsen (5) were able to produce a satisfactory blue cheese from raw, nonhomogenized milk by using a combination of heat and vacuum treatment of milk, namely 165–175° F. and 19 inches of vacuum. This treatment destroyed most of the undesirable microorganisms present in the raw milk without inactivating the milk lipase.

Irvine (8) added a commercial lipase preparation, later reported as steapsin (13), at the rate of 0.5 and 1.0 g. per 100 lb. of raw milk; the addition of the enzyme preparation resulted in accelerated fat hydrolysis and quicker ripening of the cheese as compared with the control, but a bitter flavor resulted in the cheese. Similar results were obtained by Coul-

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ter and Combs (3), who also used steapsin to hasten the ripening of blue cheese made from raw nonhomogenized milk. Thibodeau and Macy (17) added the enzymes of *Penicillium roqueforti* in the form of mycelium at the time blue cheese made from raw milk was hooped. The addition of 6 g. of mycelium to 5 lb. of cheese reduced the curing period from 10 to 5 months total time, as compared with the control without added mycelium. Parmelee (14) added pure cultures of *Alcaligenes lipolyticus*, *Achromobacter lipolyticum*, *Pseudomonas fragi* and *Mycotorula lipolytica* separately to lots of pasteurized homogenized milk made into blue cheese, and found that of these microorganisms only certain strains of *M. lipolytica* improved the flavor score and increased significantly the total volatile acidity of the cheese. This investigator also added to pasteurized homogenized milk special cultures of *P. roqueforti* grown on a modification of Czapek's medium containing 10 per cent butterfat and obtained cheese which were much superior to those made without the added special culture.

METHOD

Regular pasteurized homogenized milk of 3.5–3.8 per cent butterfat content was used in all experiments in quantities of 105 to 110 lb. per vat or lot. Three or four vats were used at one time, comprising a series, and conditions were kept as uniform as possible throughout the manufacture of cheese in each series. The vat contents were kept at 90° F. from the time of adding the culture until the curd was hooped. One per cent starter was used, and rennet was added at the rate of 90 ml. per 1,000 lb. of milk after 30 minutes holding time in series 1 to 4, and after 60 minutes holding time in series 5 to 7. Calcium chloride was added at the rate of 0.015 per cent (7 g. per 100 lb. of milk) to the milk in series 3 to 7 prior to setting. At the same time a previously standardized cell-free lipase preparation from *M. lipolytica* (16) was added in definite quantities to all but the control lot of milk in each series. The curd was cut into 0.5 inch cubes 70 minutes after setting and held for 2 hours with some stirring every 30 minutes, after which time the whey was drained. One per cent salt and 0.01 per cent mold powder were added to the curd at the time of hooping. The cheese was dry-salted daily for 4 days, using a total of 5 lb. of salt per 100 lb. of curd. Next the cheese was skewered and placed in the ripening room at approximately 10° C. and a relative humidity of approximately 90 per cent, where it remained for 12 weeks.

The cheese were examined and scored for positive flavor, defects, and visual mold growth after ripening periods of 4 and 12 weeks. A score of 10 was considered perfect in each of the three items under consideration. The total volatile acidity of the cheese was determined by the method of Lane and Hamner (10) at the ages of 4 and 12 weeks. Determinations of moisture, fat and total chlorides in the cheese at 4 weeks showed only

TABLE 1
Preliminary trials on the influence of the addition of various amounts of *M. lipolytica* lipase upon the liberation of volatile free fatty acids, flavor and mold score of ripening blue cheese

Lot no.	Amount of lipase added ^a	Vol. acidity in ml. 0.1 N acid per 200 g. of cheese		Score										Remarks on flavor at 12 weeks	
				Flavor						Mold					
				Positive		Negative									
						4 weeks	12 weeks	4 weeks	12 weeks						4 weeks
Series 1															
11	None	6.0	18 ^b	Lacking, slightly unclean	
12	100	6.6	18	Lacking, slightly unclean	
13	200	7.0	16	Lacking, sl. unclean, bitter	
Series 2															
21	None	5.4	13	Lacking, sl. unclean	
22	300	9.6	22	Sl. soapy, sl. unclean	
23	800	18.0	65	Sl. sharp, soapy	
Series 3															
31	None	7.0	13	3.0	4.0	4.0	3.5	3.5	4.0	3.5	4.0	3.5	4.0	Lacking, sour	
32	660	26.0	50	7.0	7.5	6.5	7.0	4.0	7.0	4.0	7.0	4.0	7.0	Fair, sl. sharp, sl. soapy, sl. sour	
33	1300	39.0	85	6.5	6.0	5.5	3.0	3.5	5.0	3.5	5.0	3.5	5.0	Excessively sharp and soapy	
Series 4															
41	None	9.0	18	3.5	4.0	6.0	3.5	7.5	7.5	7.5	7.5	7.5	7.5	Lacking, sl. nutty, sl. fermented	
42	300	15.0	38	6.5	5.0	6.0	4.0	3.5	6.0	3.5	6.0	3.5	6.0	Fair, sl. nutty, sl. unclean	
43	600	21.0	60	8.0	7.5	8.0	7.0	4.5	8.0	5.5	8.0	5.5	8.0	Sl. sharp, sl. soapy	
44	900	26.0	72	7.0	5.5	5.5	3.5	3.5	6.0	3.5	6.0	3.5	6.0	Excessively sharp and soapy	

^a Calculated as total lipase activity (ml. of preparation × lipase activity per ml.), the activity being expressed in acid degrees, which are defined as ml. of N NaOH required to neutralize the free fatty acids in 100 g. of fat (1).

^b No numerical score given.

TABLE 2
Further trials on the influence of the addition of various amounts of *M. lipolytica* lipase upon the liberation of volatile free fatty acids, flavor and mold score of ripening blue cheese

Lot no.	Amount of lipase added ^a	Vol. acidity in ml. 0.1 N acid per 200 g. of cheese						Score						Remarks on flavor at 12 weeks
								Flavor			Mold			
								Positive			Negative			
								4 weeks	12 weeks	4 weeks	12 weeks	4 weeks	12 weeks	
Series 5														
51	None	6.5	20.0	4.0	4.0	3.0	4.0	4.0	6.0	6.5	Lacking, musty, unclean			
52	250	10.5	27.4	5.0	4.5	4.5	5.5	6.0	5.5	2.5	Lacking, musty, sl. unclean			
53	375	10.5	33.7	5.5	6.0	6.0	6.0	5.0	5.5	5.0	Sl. lacking, sour, sl. unclean			
54	500	12.0	37.4	6.0	7.0	6.0	7.0	7.0	5.0	6.5	Fair, sl. sour, sl. unclean			
Series 6														
61	None	8.0	21.6	3.0	4.0	1.0	3.5	6.0	4.5	Lacking, bitter, musty, sl. sour				
62	250	11.0	31.2	6.0	4.5	6.0	4.5	5.0	4.5	Lacking, bitter, musty, sour				
63	375	11.0	34.6	5.5	6.0	5.0	7.0	6.0	5.0	Sl. lacking, sl. sour, sl. unclean				
64	500	13.0	35.4	6.5	6.5	6.0	6.0	5.0	7.0	Fair, sour, nutty				
Series 7														
71	None	7.0	19.5	4.0	4.5	3.5	5.0	6.0	6.0	Lacking, musty, sl. sour, fermented				
72	250	9.0	29.3	5.0	6.5	5.0	7.5	7.5	6.5	Lacking, sl. sour, sl. unnatural				
73	375	10.5	33.0	6.0	6.5	6.5	4.5	5.5	7.5	Sl. lacking, musty, unclean				
74	500	13.0	38.2	7.0	7.5	6.0	6.5	5.0	6.5	Fair, sharp, unnatural, sl. sour				

^a Calculated as total lipase activity (ml. of preparation × acid degree value per ml.) added to 105 lb. of milk.

slight variations within each series; these differences were not considered significant in the enzyme study under consideration, and therefore the data are not presented in this paper.

RESULTS

Data showing the influence of the addition of various amounts of *M. lipolytica* lipase upon the volatile acidity, flavor and mold growth of the cheese in series 1 to 4 are presented in table 1. The trials were of a preliminary nature and served to indicate the amount of enzyme required for the production of a blue cheese in which a satisfactory level of fat hydrolysis occurred. The control cheese (lots 11, 21, 31, 41) were lowest in total volatile acidity in their respective series and were lacking completely in the desired ketone flavor characteristic of properly ripened blue cheese. Additions of *M. lipolytica* lipase to the milk resulted in increases in the total volatile acidity of the cheese in proportion to the amount of lipase added. Cheese with total volatile acidity values of 50 and above at 12 weeks were criticized for being soapy and sharp, both characteristics being undesirable (lots 23, 32, 33, 43, 44). Lots 32 and 43 were most satisfactory from both body and flavor standpoint, although they also were criticized for being slightly soapy and slightly sharp.

Table 2 shows the results of replicate series 5, 6 and 7 made within a 5-day period after the complete data of the first four series had been collected. Again the total volatile acidity values of the controls (lots 51, 61, 71) were the lowest in each respective series, with the values increasing in the order of increasing enzyme concentration of the cheese. A close correlation existed between the total volatile acidity values of the cheese in the three series and the concentration of enzyme used. The flavor score, and to a certain extent also the defect score, showed good correlation with the total volatile acidity values, highest scores being given to lots 54, 64 and 74 which showed total volatile acidity values at 12 weeks of 37.4, 35.4 and 38.2, respectively. None of the cheese in these series was criticized for soapiness or excessive sharpness, although other defects were encountered; however, these could not be attributed to the enzyme added. This was also true in the first four series (table 1).

There was no indication in the cheese of any one of the seven series that mold growth was affected by the different amounts of total volatile acidity present at any time in the individual lot of cheese. No correlation could be established between mold score and flavor score of any one cheese. Although the mold scores of the different cheese varied from 4 to 7.5, all of the cheese showed sufficient mold growth to permit flavor development if other conditions were satisfactory.

DISCUSSION

The addition of *M. lipolytica* lipase to pasteurized milk which then was made into blue cheese brought about the desired hydrolysis of the fat.

The acidity of the cheese and the temperature at which the cheese was ripened both were favorable for the action of the lipase, as had been anticipated from previous study of this enzyme system (16). A good relationship existed between the amount of enzyme added and the values for total free volatile fatty acid obtained at 4 and 12 weeks of ripening of the cheese. The cheese containing the added lipase had more organoleptically detectable free fatty acids, as well as ketone flavor, and a waxier body than the control cheese without added lipase. These observations suggest that the lipase added was of considerable value in aiding in the proper ripening of blue cheese. According to Lane and Hammer (10), a satisfactory ripened blue cheese was not obtained until after 16 weeks holding time, when pasteurized, homogenized milk was used, while with raw homogenized milk a satisfactory ripened cheese was obtained in 12 weeks. Thus the presence of lipase, either milk lipase or added lipase such as used in this study, brings about early hydrolysis of the fat and thus enables the mold to utilize the free fatty acids and to change certain ones into flavor-producing ketones (7).

The sharp, soapy taste in a number of cheese was correlated with free fatty acid values of 50 and higher in the cheese after ripening for 12 weeks. Cheese with most desirable flavor at this age showed values between 30 and 50. Other workers have made observations which support this conclusion (10, 14). The flavor of the cheese containing the added lipase, while characteristic of blue cheese, did not duplicate exactly the flavor of the product made from homogenized raw milk. However, most of those who sampled the cheese made from pasteurized homogenized milk containing the added lipase accepted the product as satisfactory cheese with a high level of good flavor development. Under no circumstances was a bitter or other objectionable flavor definitely attributable to the addition of the microbial lipase. Less breakdown of the body to a desirable level was observed in the controls than in the cheese made with added lipase. Since *M. lipolytica* is both lipolytic and proteolytic, it is possible that the cell-free lipase preparation also carried some proteolytic enzymes which were beneficial to the breakdown of the protein in the cheese. No data were collected on this phase of cheese ripening, although a study of this point would be desirable.

The repeatedly observed close relationship between total activity of lipolytic enzyme preparation added to the milk and extent of fat degradation in the resulting cheese permitted the addition of predetermined amounts of enzyme which would result in the desired level of total volatile acidity in the cheese after the ripening period of 12 weeks employed in this study.

The data indicate that cell-free lipase obtained from cultures of *M. lipolytica* could be used to advantage in the manufacture of blue cheese

made from pasteurized homogenized milk, and possibly also in other varieties of cheese in which hydrolysis of fat is essential for the proper ripening of the cheese.

SUMMARY AND CONCLUSIONS

1. Seven series of blue cheese were made from pasteurized homogenized milk with and without the addition of a cell-free lipase preparation obtained from *Mycotorula lipolytica*.

2. Examinations of the cheese at 12 weeks for flavor and other desirable characteristics showed the cheese ripened with the aid of the cell-free lipase preparation consistently was more satisfactory than the corresponding control containing no added lipase.

3. Increases in the concentration of lipase in the cheese resulted in increases in total volatile acidity values of the cheese and also of the intensity of the flavor typical of blue cheese. Cheese with enzyme concentrations high enough to show total volatile acidity values of from 30 to 50 after ripening for 12 weeks were most satisfactory in flavor. Cheese with total volatile acidity values above 50 were criticized as being sharp and soapy in every case.

4. The results of this study indicate that the cell-free lipase prepared from cultures of *M. lipolytica* can be used advantageously in the ripening of blue cheese made from pasteurized homogenized milk.

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MOISTURE STUDIES IN DRY PRODUCTS OF MILK. II. ESTIMATING WATER OF CRYSTALLIZATION OF ALPHA-LACTOSE IN DRY WHEY SOLIDS

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In the previous paper on the kinetics of moisture desorption of crystalline *alpha*-lactose hydrate (1), a possible method was suggested for estimating the water of crystallization of lactose in dry products of milk. In the present communication experimental evidence in support of the method, together with results obtained on dry whey solids, is presented. Dry whey solids have been selected for study, because samples with the lactose largely in the form of the crystalline hydrate are readily available.

The lactose in nonfat dry milk solids and dry whey solids manufactured by the ordinary spray and roller processes has been reported to be amorphous (5, 7). In recent years, however, various processes have been developed for inducing crystallization of lactose as the *beta*-anhydride or as the *alpha*-hydrate in dry whey solids (5).

Sharp *et al.* (6) have found that the state of lactose in dry products of milk has a great influence on the determination of moisture by the toluene distillation method. For products containing crystalline lactose hydrate, a longer distillation is necessary than for similar products in which the lactose is in the amorphous state. Presumably, the loss of moisture at the later stage is due to the dehydration of crystalline *alpha*-lactose hydrate. In a previous study on crystalline *alpha*-lactose hydrate in boiling toluene (1), this laboratory observed that the rate of dehydration follows the first order kinetics expression,

$$k = \frac{2.303}{t} \log \frac{a}{(a-x)}$$

where k is the rate constant, a the initial moisture content, and x the amount of moisture removed in time t . Therefore, it appears possible to estimate the water of crystallization of *alpha*-lactose and consequently crystalline lactose hydrate by taking advantage of this difference in the rates of moisture removal and of the unimolecular character of the dehydration of crystalline lactose hydrate.

EXPERIMENTAL PROCEDURE

Moisture desorption method. The apparatus used was exactly the same as that employed previously in the study on crystalline *alpha*-lactose hydrate (1). Fifty grams of sample were weighed into the 300-ml. Erlenmeyer flask and quickly covered with 100 ml. of moisture-free toluene. After at-

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taching the flask containing the sample to the apparatus, toluene was added through the top of the condenser to fill the moisture trap. Stirring then was applied to keep the mixture well agitated. The rate of distillation was adjusted to give more than two drops per second (1). At 5- or 10-minute intervals after the first appearance of moisture in the trap, the volume of water collected was read and multiplied by two to convert to per cent of

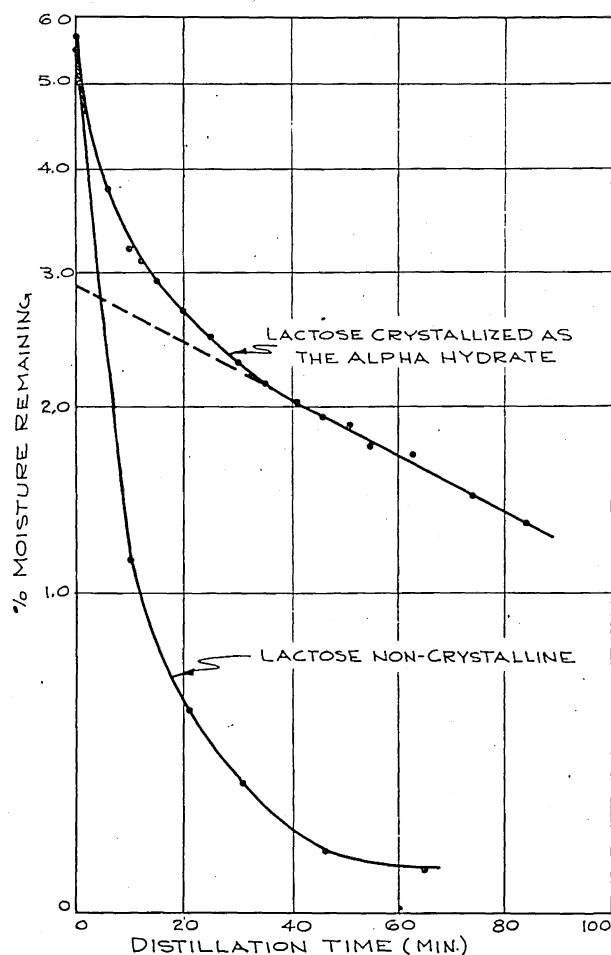


Fig. 1. Typical moisture desorption curves of two different types of dry whey solids.

water desorbed. The total moisture was determined by the Karl Fischer method (2). Less accurately, it may be estimated by continued distillation until a sufficiently constant value is obtained; this generally requires about 3 hours for the type of dry whey solids studied. The logarithm of the per cent moisture remaining in the sample, *i.e.*, $\log(a-x)$, at each time interval was plotted against the distillation time in minutes. The top curve (fig. 1) has two portions. The initial steep portion represents the dehydration of

crystalline *alpha*-lactose hydrate together with the protein hydrates and surface moisture. The second portion is linear and represents the dehydration of crystalline *alpha*-lactose hydrate. Consequently, by extrapolating the straight-line portion to zero time, the initial percentage of water from crystalline *alpha*-lactose hydrate may be obtained by taking the anti-logarithm of the vertical intercept. If desired, the percentage of crystalline *alpha*-lactose hydrate present in each sample can be obtained by dividing the determined per cent of water of crystallization by 0.050.

Indirect method. The indirect method referred to in table 1 is a combination of two determinations: (a) total moisture by the Karl Fischer pro-

TABLE 1
Water of crystallization of alpha-lactose in some dry whey solids by two methods

Sample no.	Indirect method			Desorption method
	% Total H ₂ O (Karl Fischer)	% Free H ₂ O (vac. oven)	% H ₂ O crystallization	
1	5.68	2.73	2.95	(%) 2.89
2	5.31	2.55	2.76	2.70
3	5.44	2.82	2.62	2.73
4	5.27	2.48	2.79	2.89
5	5.79	3.08	2.71	2.52
6	4.47	1.89	2.58	2.60
7	5.43	2.68	2.75	2.67
8	4.91	2.00	2.91
9	3.88	1.23	2.65	2.69
10	5.76	3.10	2.66	2.95
11	4.21	1.71	2.50	2.62
12	3.81	1.38	2.43	2.40
13	4.33	1.84	2.49	2.51
14	3.70	1.30	2.40	2.45
15	3.67	0.87	2.80	2.88
16	4.93	2.13	2.80	2.94

cedure of Fosnot and Haman (2) using visual end-point estimation and (b) "free" moisture by dehydration of a 4-g. sample in a Cenco-DeKhotinsky vacuum oven at 65° C. and 2-3 mm. mercury pressure for 5 hours (4). This method again is based upon the fact that lactose hydrate dehydrates at an extremely slow rate under the conditions used in the determination of "free" water. Thus, in two experiments with crystalline lactose hydrate of particle sizes less than 149 μ , only 0.05 and 0.07 per cent of moisture were removed in 5 hours. On the other hand, dry casein containing approximately 8 per cent moisture appeared to be completely dehydrated. The difference between the Karl Fischer result and that obtained in the low temperature vacuum oven determination was inferred to be water of crystallization of *alpha*-lactose.

Samples. All samples of dry whey solids used in this study were selected from samples currently sent to this laboratory for analysis. The particle

sizes of these products generally are within the range of about 54 to 210 μ . Crystalline *alpha*-lactose hydrate was Baker's C.P. powder containing the theoretical 5.0 per cent water of crystallization as determined by the Karl Fischer method (2). The particle sizes were under 149 μ . Dry casein was of technical grade obtained from J. T. Baker Chemical Company.

RESULTS

Figure 1 shows two typical moisture desorption curves for two different types of dry whey solids. For the top curve the lactose in the product is

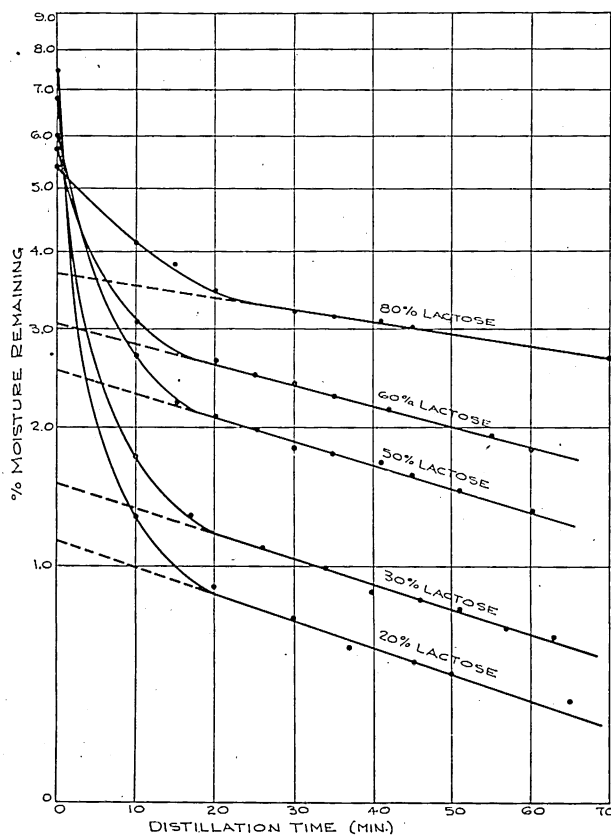


Fig. 2. Dehydration curves of mixtures of *alpha*-lactose hydrate and casein.

present largely in the form of the crystalline *alpha* hydrate, as indicated by the seeding test used by Troy and Sharp (7). This curve illustrates the initial rapid loss of moisture and the slower constant desorption after the first 20-30 minutes. For the lower curve the lactose is in the glass or amorphous state, as shown by a negative seeding test. This type of dry whey solids forms a single hard mass in boiling toluene and, in spite of the resultant reduction of surface area, shows a rapid rate of dehydration.

Since protein and lactose are the two major constituents in most dry products of milk, the method was applied to mixtures containing different proportions of dry casein and lactose hydrate to see how well the latter can be recovered. Results are plotted in figure 2. The casein used for the first two trials contained approximately 7 per cent moisture and was less than $149\ \mu$ in particle size. The remaining trials were conducted with dry casein of slightly higher moisture content and of particle size less than $210\ \mu$.

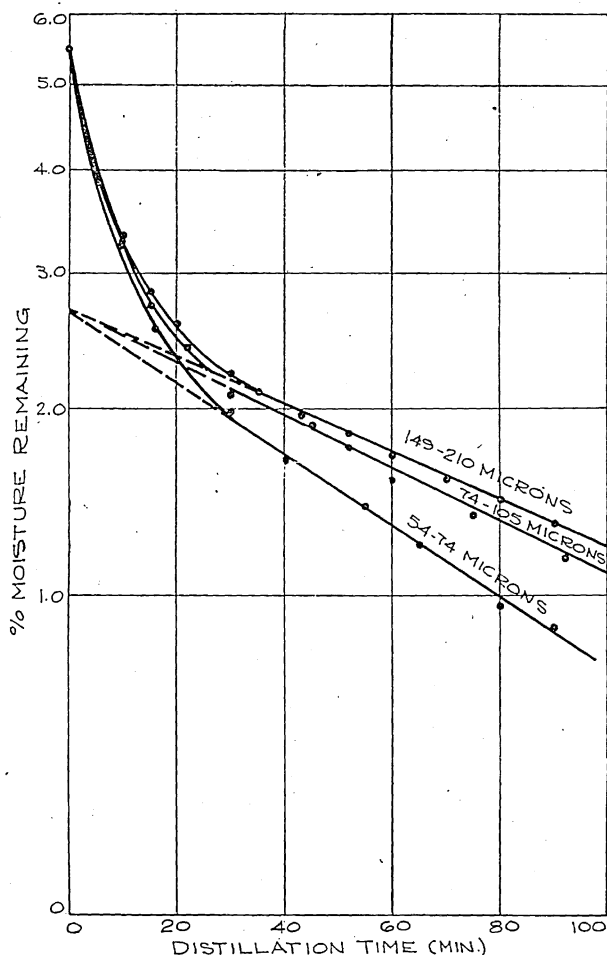


FIG. 3. Effect of particle size on the determination of *alpha*-lactose hydrate in dry whey solids.

A comparison of results by the desorption method with those by the indirect method on samples of dry whey solids which gave positive seeding tests for crystalline *alpha*-lactose hydrate is presented in table 1. Under conditions employed in the vacuum oven test for "free" moisture, weight loss after 5 hours of heating was constant.

In order to determine whether particle size has any effect on the final result, a sample of dry whey solids was fractionated into different particle sizes by means of standard sieves. Results are plotted in figure 3. The straight lines used for extrapolation were calculated by the method of least squares.

DISCUSSION

The difference in moisture desorption behavior of the two types of dry whey solids is quite evident from figure 1. It can be seen that the presence of crystalline *alpha*-lactose hydrate gives rise to a slower rate of desorption, which is unimolecular at the later stage. Since the composition of the two types of dry whey solids is approximately the same, it is unlikely that this difference in the rate of desorption could have arisen from any other sources.

The results of the experiments using crystalline *alpha*-lactose hydrate and dry casein, as shown in figure 2, indicate that at least in simple mixtures of the two materials, water of crystallization of lactose can be quantitatively differentiated from water adsorbed by casein. Moreover, the similarity of curve 1 shown in figure 1 to those in figure 2 tends to support the previous interpretation of each portion of the curve.

The apparent agreement between results obtained by the moisture desorption method and the indirect method as shown in table 1, in all probability, is not accidental. Admittedly, both methods are based upon the slowness in the dehydration of crystalline *alpha*-hydrate as compared with other moisture adsorbing constituents. Yet the two methods differ entirely in other respects. Whereas one method determines water of crystallization of lactose hydrate from the difference between total and "free" moisture, the other depends upon the unimolecular character of the dehydration of crystalline lactose hydrate in boiling toluene. Agreement between the two series of results must be considered good in view of the fact that the moisture determinations by even the best available methods usually involve deviations of the magnitude of 0.1-0.2 per cent.

Referring to figure 3, particle size within the range studied does not seem to have any influence on the extrapolated value in the desorption method aside from changing the rate of dehydration. Ideally, a sample should be homogeneous with respect to particle size. Practically, it has been found that for particle size occurring normally in dry whey solids of the type studied, the linear relationship for the dehydration of crystalline *alpha*-lactose hydrate still is obeyed.

From the above evidence it appears that both the moisture desorption method and the indirect method can be used for estimating water of crystallization of lactose and consequently of *alpha*-lactose hydrate itself in certain dry whey solids. Presumably, the methods can be applied to other dry products of milk containing crystalline lactose hydrate. It must be

pointed out that the desorption method depends on the continued presence of crystalline *alpha*-lactose hydrate after complete removal of all other forms of moisture. For this reason the method may not be as accurate for products containing small quantities of crystalline *alpha*-lactose hydrate as for the dry whey solids studied.

SUMMARY

A moisture desorption method has been developed for estimating the water of crystallization of *alpha*-lactose and indirectly the crystalline *alpha* hydrate itself in certain types of dry whey solids. It is based upon the difference in the rates of dehydration of crystalline *alpha*-lactose hydrate and other moisture adsorbing constituents and also upon the unimolecular dehydration of the hydrate itself.

Good recovery was obtained using mixtures of known composition of crystalline *alpha*-lactose hydrate and casein.

Results obtained by this method were in close agreement with those by an indirect method in which the difference between total moisture as determined by the Karl Fischer method and "free" moisture as determined by an oven procedure was considered to be water of crystallization of *alpha*-lactose.

Aside from an effect on the rate of dehydration, particle size, within the range of 54 to 210 μ , was found to have no influence on the results obtained by the moisture desorption method.

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THE NUTRITIVE VALUE OF HOMOGENIZED MILK: A REVIEW¹

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The consuming public seems to have accepted homogenized milk far beyond the anticipations of the early enthusiasts for the product. In the beginning, extravagant claims for the virtues of homogenized milk sometimes were made, mostly in good faith, to arouse interest in it. Once the consumer gave properly processed homogenized milk a fair trial, he generally became an enthusiast for the new product. In fact, homogenized milk once used often sold itself because of certain inherent qualities, and the extravagant claims were soon forgotten or ignored. Nevertheless, the belief persists in some sections that homogenized milk has superior nutritive values, thus presuming that nonhomogenized milk is lacking somewhat in those properties or values which give the full measure of nutrition. Consequently, the evaluation of the contributing factors affecting the nutritive value of homogenized milk in the light of scientific data on the subject seems desirable. Among the factors which may contribute to better nutrition when homogenized milk is used and which should be reviewed are: (I) palatability; (II) homogeneity; (III) curd tension; (IV) digestibility; and (V) miscellaneous factors. Also, it seemed expedient to review its use in infant feeding (VI) as well.

Extravagant Claims for Homogenized Milk

The early attempts to introduce homogenized milk in America in the Province of Quebec, Canada, around 1909 were not successful. Nearly 20 years were to pass before the public accepted the product. It should be pointed out, however, that although at both periods the milks were called "homogenized," there was, in reality, a considerable difference between them. The homogenized milk introduced in 1909, rendered homogeneous by means of the Gaulin homogenizer, was sterilized in bottles at 226° F. for 20 minutes; that of 1927 was properly homogenized but was not a sterilized product. The homogenized milk of 1909 was heralded as a medicinal product, a "cure all"; that of 1927 was recognized merely as a milk of good flavor. For example, in 1909, Trudel (115) made the following therapeutic claims for homogenized milk:

¹ Journal Article no. 944 (n.s.) from The Michigan Agricultural Experiment Station.

“‘Laurentia’ (homogenized) milk, especially the *maternized* (homogenized and sterilized)² constitutes a food of the highest order, and is particularly to be recommended for bottle babies, in cases of diseases of the digestive tube, such as gastroenteritis, green diarrhoea, cholera infantum and also for children suffering from ringworm of the scalp or face.

“The use of ‘Laurentia’ milk is also advisable for adults suffering from dyspepsia, acute or chronic gastritis, irritation of catarrh of the stomach consequent upon dietetic (*sic*) excesses or the *abuse* of alcoholic liquors. It also constitutes a valuable food for persons afflicted with kidney and liver diseases such as congestion of these organs, jaundice, cyrrhose (*sic*), acute or chronic nevritis (*sic*), Bright’s disease, as well as in cases of inflammation of the bladder and the urinary canal. Finally, it is to be recommended in cases of infectious diseases, where the system already invaded by microbes and their secretions needs a perfectly aseptic food, under this heading we may include measles, scarlate (*sic*) fever, diphtheria, grippe, typhoid fever, etc., and also all kinds of poisoning, acute or chronic.”

Thus, it is not surprising that early attempts to sell homogenized milk were not successful. Unfortunately, even after a lapse of over thirty years, some extravagant claims for homogenized milk still persist, so that Harding (52) in 1938 was impelled to write as follows:

“Homogenization has suffered more from its friends than from any other cause.

“They commonly say that homogenized milk digests much better than the untreated milk. As a matter of fact, milk is one of our most completely digested foods and much of this talk in favor of homogenization has been little less than a libel against the ordinary milk.

“It is hard to understand why some milk dealers will make such an unfair presentation regarding their ordinary milk in the hopes of selling a few bottles of homogenized milk. If the statements were true it would be a doubtful business procedure, and when there seems to be an entire lack of dependable data for the statement that homogenization makes any measurable increase in the digestibility of milk the business motive is hard to understand.”

Advantages Reported for Homogenized Milk

Buttenberg (21), Pittius (88), Bordas *et al.* (16), Schoofs (92), Bishop and Murphy (13), and von Sobbe (94) early pointed out that the prevention of the removal of cream was one of the major advantages of homogenized milk. Sidersky (93) believed that the fresh milk taste of homogenized milk was a factor in its favor, as well as the stability of its fat emulsion. Istaz and Van Soest (64) stated that homogenized milk had a very fine flavor and was especially well suited for nutrition. Gaulin *et al.* (47) demonstrated to their own satisfaction that homogenized milk was “much more digestible” than nonhomogenized milk. In addition, Bishop and Murphy (13) reported claims that homogenized milk was more easily digestible.

More recently, Hess *et al.* (53) believed that the “greatest value of all these milk modifications (heat treatments and additions) may lie in the

² Reviewer’s explanation.

soft, broken curd, which exposes a large surface to the digestion juices and to denaturization of the proteins." Jones (70) emphasized that when using homogenized milk every consumer would receive his fair share of milk fat since there could be no stealing of cream, leaving the skim milk for the children. He admonished, "Homogenized milk can never be skim milk." With wider use of homogenized milk, other nutritional advantages were claimed for it.

Wolman (127, 129) believed that "the nutritive benefit of adequately homogenized milk—whether pressure treated or sonized—rests in the small soft curds that form in the stomach of the individual who drinks the milk. Certain other advantages might be mentioned, namely, improved flavor, uniform distribution of the fat, and protection against theft of cream content." Later he (132) pointed out that the sanitary quality of homogenized milk was as important as its curd quality, stating that a low bacterial count could be maintained with higher-than-normal holder pasteurization temperatures without producing a detectable heated flavor.

Of 22 reasons classified by Tracy (105) from consumers as to why they used homogenized milk, 11 seemed to have a direct or indirect bearing upon its nutritive value. These were:

- a. "Tastes richer."
- b. "Easy to prepare for baby feeding."
- c. "The last glass is as good as the first."
- d. "No cream rises to top of glass standing in refrigerator."
- e. "There is no temptation to remove the cream."
- f. "There is no loss of cream in scum after boiling."
- g. "Feedings prepared for infants in advance do not have to be remixed when used."
- h. "Our youngsters refuse to drink any other kind of milk."
- i. "As a result of my children's drinking more of this milk, all have gained weight."
- j. "Nipples on bottles do not stop up and baby gets proper proportion of fat."
- k. "An infant born in October was fed prepared powdered milk but was unable to properly digest fat. Was then fed homogenized milk which has been digested easily and infant has been gaining weight steadily."

Wolman (131, 133) listed four advantages of homogenized milk of medical interest and importance when compared with ordinary market milks, namely: (a) uniform distribution of fat; (b) good flavor; (c) possibility of high sanitary quality; and (d) altered curd properties resulting in improved digestibility.

McLester (84) gave the following advantages for homogenized milk:

"It is more easily digested; because of the smaller size of the fat droplets to

which it is attached, the vitamin D is better utilized; the fat globules do not rise to the top but remained suspended, and the curd is smaller and more easily digested."

Another advantage of considerable merit is its suitability for infant feeding (33, 43, 46, 66, 69, 111, 127, 129, 130, 131), and in hospitals (33, 127).

A nutritional advantage of the use of homogenized milk in hospitals, institutions and restaurants is well stated by Doan (34) as follows:

"The process is of particular advantage where the milk is to be dispensed from bulk as is the case in hospitals, institutions, restaurants, etc. It will insure that each recipient of the product obtains a proper proportion of the fat and the 'cream' is not kept in the kitchen for benefit of the 'help'. In hospital use it is preferred by the dietitian because it is certain that each portion of the milk is identical and that one patient does not get 'light cream' and another skim milk."

The use of homogenized milk in schools would seem to be of a direct advantage. Kelly (71) found that when nonhomogenized milk was served to school children in half-pint bottles, an average of 5.6 per cent of the milk was left in the bottle. This remnant contained nearly 16 per cent of the fat. Obviously, the milk was not thoroughly mixed before serving; nor could the children be relied upon to do the mixing. Evidently when the straws were inserted, the bottom layer was drawn off first, while the creamy portion remained until the last. These data indicated that the school children were being deprived of a portion of the butterfat they were supposed to have. He concluded that this loss could be largely obviated by proper homogenization of all milk delivered to schools.

I. PALATABILITY

Palatability is an important attribute of foods, having an indirect bearing upon nutrition. Unless food is palatable it will not be readily accepted or ingested with anticipation. Moreover, it is reasonable to presume that larger quantities of palatable than of non-palatable foods will be consumed. Several factors, such as initial flavor, stability of flavor, homogeneity, appearance, smoothness, and absence of cream clots (93), influence the palatability of milk. Properly processed homogenized milk has those properties, but as pointed out by Hood and White (61), homogenized milk is more susceptible to an off-flavor due to light, the activated or sunshine flavor, than nonhomogenized milk.

That the early homogenized milk was not always palatable is not surprising. The early process involving sterilization of the milk yielded a cooked flavor. Furthermore, the role of lipase in decreasing the pH (32) and inducing lipolysis (42, 51) was not known. Despite the fact that increased acidities were encountered in early homogenization (16) and that much discussion was had in 1905 over a "bad" flavor, resembling butyric acid, which was believed to be due to the contamination and growth of specific bacteria in homogenized milk (47), Ladd (77) reported that homo-

genization decidedly improved the flavor of milk. However, Washburn and Jones (121) reported that homogenized milk was not as palatable as nonhomogenized milk. They observed that pigs apparently disliked homogenized milk. They noted that throughout the feeding trial the pigs which were fed homogenized milk were not inclined to gorge themselves and that the homogenized milk was not relished as well as the normal milk, although the animals to which it was fed had never been accustomed to cow's milk. It must be pointed out, however, that the milk was raw, was homogenized at 85° F., and was stored for some time—conditions favoring development of rancidity. Without a doubt this homogenized milk was so rancid and repulsive that even pigs drank it reluctantly. On the other hand, Berry (11) found that rats receiving homogenized milk consumed 17.5 ml. more milk per day per rat on the average than those having access to normal hard-curd milk. Tretsen (108) found, also, that rats consumed more homogenized milk than normal hard-curd or normal soft-curd milk.

In comparing the flavor of nonhomogenized and homogenized milk, the flavors of the fresh products and those of samples stored for several days following processing should be considered. Homogenization does fix the clean, sweet flavor of milk against oxidation (107).

Babcock (6), securing 470 opinions on 470 samples each of homogenized and nonhomogenized milk as to its palatability, found that with milk of good flavor, properly homogenized, 62.1 per cent of the opinions showed no prejudice against homogenized milk. He (7) believed that the improved palatability reported for properly prepared homogenized milk might make it an important factor in increasing the per capita consumption of milk.

Trout (110) and Trout *et al.* (113), comparing the flavors of freshly processed products, noted that: (a) homogenization of pasteurized milk did not impair the flavor or cause the development of any undesirable flavor; (b) the flavor of homogenized pasteurized milk did not merit first choice over the nonhomogenized milk by the majority of the judges; (c) difficulty was encountered in picking out the homogenized samples from the nonhomogenized; and (d) the homogenized milk seemed smoother, and therefore, richer to some of the judges than the nonhomogenized pasteurized milk.

Doan (37), making a preference study, found that a greater number of people preferred homogenized to nonhomogenized milk at all fat levels from 2.5 to 5.0 per cent at 0.5 intervals but noted the highest percentage of preferences was obtained at the 4.0 per cent level. The consensus was that the process made the milk "richer", "smoother" or "creamier".

Homogenization retards or inhibits the development of the oxidized flavor of milk, as shown by Tracy *et al.* (107), by Thurston *et al.* (103), and later substantiated by Ross (89), Doan (35), Trout and Gould (112),

Larsen *et al.* (78), and Babcock (8). Thus, properly stored homogenized milk retains the fresh flavor over an extended period, a factor not to be discounted in evaluating the flavor of homogenized milk. Moreover, homogenized milk cannot be mixed or blended with raw milk and retain its palatability, for such mixtures soon become rancid (42, 49, 79).

Wolman (130) observed that homogenized cow's milk had a pleasing flavor. He (131) believed that the masking effect of homogenization on the possible "cooked" flavor when milk was pasteurized at 150° F., as shown by Spur (96), was beneficial in that the flavor was not impaired and the augmented pasteurization heat was of obvious importance as a sanitary safeguard.

II. HOMOGENEITY

Properly processed homogenized milk retains its homogeneity over an extended period of time (93). This property early was recognized in the product. In fact, such milk originally was known as fixed milk, "lait fixe" (47), and the process itself was known as "fixation" (16, 92). That the product could not be creamed or churned in transport was the basis for the prediction of a favorable future for homogenized milk (93). Despite absence of cream-line formation on properly processed homogenized milk (106, 114), some upward migration of fat globules occurs upon long standing (40, 114), and some settling of the fat and solids-not-fat occurs when the milk is frozen and thawed slowly (110). Nevertheless, the actual upward migration of fat in a quart of milk is practically insignificant, slightly over 1 per cent, even when the United States Public Health Service maximum of 10 per cent differential between the percentage fat in the upper 100 ml. and the remainder of the quart has been reached. Thus, for all practical purposes, properly homogenized milk is virtually homogeneous throughout.

Distribution of vitamin A and vitamin D. Vitamins A and D, associated with milk fat, are distributed uniformly throughout properly homogenized milk and thus are available proportionately as the milk is consumed. There is no removal of the vitamin A- and D-bearing fat of homogenized milk in the form of cream for coffee, leaving the less-vitamin-rich milk for beverage use. Jeans (65) believed that when vitamin D was added to milk, its even distribution with the cream throughout the milk by means of homogenization was advantageous. Thus, homogenized milk appears to be the best possible vehicle for added vitamin D.

Marriott and Jeans (81) advised that in homogenized, vitamin-D milk the vitamin D was evenly distributed and, when the milk contained 400 units to the quart, the vitamin D intake of the baby was ample if customary amounts of milk were ingested. Krauss *et al.* (75), making bioassays by the line-test procedure of normal milk, homogenized milk and mineral

modified soft-curd milk, all from the same source, and natural soft-curd milk, all fortified to the extent of 400 units of vitamin D per quart, found the same degree of healing when equal amounts of each were fed. They concluded that the effectiveness of added vitamin D was not influenced by the curd-tension of the milk. McLester (84) believed that the vitamin D of homogenized milk was better utilized because of the smaller size of the fat globules to which it was attached.

III. CURD TENSION

There are many ways of lowering the curd-tension of cow's milk, such as acidifying, diluting, boiling, mineral modification, enzyme treatment and homogenization. Apparently, however, homogenized milk is the soft-curd milk most widely sold (132). In fact, homogenized milk often is advertised as soft-curd milk, thus featuring this property to the exclusion of other important factors bearing upon its nutritive properties.

As early as 1916 Washburn and Jones (121) noted that curds formed from homogenized milk were so much more flocculent and friable as a result of this process that these workers were led to believe that benefit might be expected from such treatment.

Not until 1923 when Hill (54) developed a test for determining the curd tension of milk, believing that the curd variance might be an index to the food value of milk for infants, was there much interest shown in soft-curd milk.

Since then many research workers (2, 10, 11, 22, 23, 33, 41, 75, 80, 86, 90, 102, 104, 105, 106, 113, 119, 120, 122, 123) have noted a lowering of the curd tension of milk as a result of the homogenization process.

Percentage reduction. Wallace (119) reported that homogenization reduced the curd tension about 50 per cent; Doan and Welch (41) found that proper homogenization reduced the curd tension to about 40 per cent of that of the original milk; while Theophilus, *et al.* (102) observed that homogenization at pressures of 500, 1,000 and 2,000 lb. reduced the curd tension of milk approximately 25, 46 and 53 per cent, respectively. Krauss *et al.* (75) noted that homogenization at 2,500 lb. pressure reduced the curd tension 61.5 per cent.

Essential pressures. Berry (11) concluded that homogenization pressures of 3,000 to 5,000 lb. were required to render hard-curd milk (50 to 112 g.) a soft-curd milk. The higher the curd tension of the original milk, the greater was the percentage reduction of curd tension after homogenization. Tracy (104, 106) showed that the curd tension of homogenized milk varied with the season and with the temperatures and pressures of homogenization and emphasized the importance of regulating the homogenizing processes so as to get as low curd tension as possible. He (105, 106) secured approximately maximum reduction in curd tension of milk

when homogenizing at 2,500 lb. pressure. Likewise, Caulfield and Martin (22), Doan (35), and Babcock (8) observed that pressures in excess of 2,500 lb. per square inch appeared to be of little practical value in further reducing the curd tension of milk.

Chambers (23) noted in sonic vibration studies that a direct relationship existed between the degree of fat dispersion and degree of curd tension reduction.

Jeans *et al.* (67) stated that homogenization gave soft-curd character to milk when the pressure and temperature were appropriate. Later Marriott and Jeans (81) advised that milk homogenized at pressures of 2,500 to 4,000 lb. to the square inch would have a curd tension less than 20 g.

Mechanism of the lowering of the curd tension of milk by homogenization. Lundstedt (80) attributed the curd softening effect of homogenization to the increased fat surface which "will remove up to 25% of the casein from the serum by the phenomenon of adsorption. The reduction in the concentration of the available casein results in a lowered curd tension." Evidently these statements are based upon the findings of Wiegner (124) to the effect that in the case of normal milk 2 per cent of the total casein was adsorbed on the surface of the fat globules, whereas with homogenized milk 25 per cent of the casein was adsorbed on the fat.

On the other hand, Sommer (95) explained that the effect of homogenization on curd tension was attributable to the increase in the number of globules serving as points of weakness in the coagulum and to a lesser extent to the casein adsorption on the increased fat surface area causing a lower concentration in the serum proper.

Nature of curd of homogenized milk. Not only is the curd tension of milk changed by homogenization, but its character is changed as well (116). Associated with the soft texture are smaller curds, thus increasing the total surface area (8, 130, 131).

Anthony (5), examining curds from homogenized milk retained in the human stomach 0.5 hour and then regurgitated, found them to be fine, uniform, soft, porous and permeable. Prior to this, however, Gaulin *et al.* (47) had reported with conviction as follows: "Fixed milk is much more digestible and I proved this by comparing homogenized milk with nonhomogenized milk. I placed in each vat a drop of the same rennet: the nonhomogenized milk gives a very thick curdled milk, hard as rubber; the other, on the contrary, a very divided curdled milk resembling the whites of eggs beaten like snow; obviously, this latter must be much more digestible than the former."³

Curd-surface area. When the average curd tension of raw milk (46 g.) was reduced to 10 g. by homogenizing and pasteurizing, the average curd surface increased approximately 230 per cent (73). When a number of

³ Literal English translation from the original.

determinations were averaged, there appeared to be a rather definite inverse correlation between curd tension and curd area (74).

Storrs (100) observed that with untreated, homogenized, enzyme-treated, and base-exchange milks studied *in vitro* at pH ranging from 6.0 to 4.0, the amount of curd recovered from homogenized milk varied the least and tended to be somewhat bulkier than that of the other milks throughout the entire pH range. He (101) found no significant relationship between curd tension and curd surface area and believed that these characteristics were independent, each being influenced or determined, possibly, by factors not closely related.

However, Babcock (8) showed that, on the average, as the curd tension of milk was lowered by homogenization, the surface area of the curds increased, but the increases were not significant from the digestion standpoint until the milk was homogenized at a pressure of 2,000 lb. or more.

Spur and Wolman (99) found that the curd area (curd number or index) paralleled the curd tension; the homogenized milks, being soft-curd, yielded the higher curd numbers. Spur (98) noted that the curd numbers of commercial homogenized milks were considerably above 200 and consequently showed no large curds at all.

Soft curd of commercial homogenized market milk. The "softness" of the curd of commercial homogenized milk sometimes is questioned, with the inference that the milk is not properly homogenized with respect to pressure and temperature. The Council on Foods of the American Medical Association (2) pointed out that unless the conditions of processing were known to be suitable, the fact that the milk was homogenized was no assurance that the milk was soft curd.

Likewise, Chambers (24) tested homogenized milks in which there was quite satisfactory fat dispersion without any decrease whatever in curd tension, and without perceptible alteration in the curd size or texture. He (25) reported that "in many localities where several brands of homogenized milk are being sold on the same claim of enhanced digestibility, tests have shown that some actually show change in curd properties while others show no change whatever. These differences are usually traceable to improper maintenance of apparatus, inadequate homogenization pressure, improper temperature control, or other defects in plant operation over which there is at present no control."

Kugelmass (76) also warned that mere homogenization was no assurance that soft-curd milk had been produced unless the conditions of homogenization had been determined.

Nevertheless, Spur (97) found that the curd tension of grade A and grade B homogenized milks marketed by 36 dairies in Philadelphia averaged 11.6 and 11.2, respectively, and ranged from 5.3 to 18.4 g. and from

4.6 and 16.4 g., respectively. These values are well under either the 20 or the 30 g. standard.

IV. DIGESTIBILITY

As pointed out by Harding (52), many extravagant claims have been made for homogenized milk. Those probably recurring most frequently have a bearing upon its digestibility. Chambers (24) believed there had been altogether too much indiscriminate talk about the improved digestibility of homogenized milk, with the result that homogenization has become synonymous with ease of utilization. Such references as 'more completely digestible', 'more easily digested', 'more rapidly digested' and 'improved digestibility' appear to be more often the result of general observation and postulation rather than the result of controlled experimentation.

Chambers (24) believed that the design of satisfactory tests for digestibility of milk was difficult because of our incomplete knowledge of the sequence of events in the human digestive system. He pointed out that two possibilities existed, namely: (a) feeding the processed milk over a period of weeks or months to representative groups of normal children, and (b) chemical or physical tests of the milk which could be shown to correlate with the effect of feeding the product to infants. Of the latter, curd tension, curd size and *in vitro* digestion have been employed extensively.

Ease of digestibility of homogenized milk. Early speculation (4, 77) was made on the influence of homogenization on the digestibility of the milk, since the curd appeared soft and friable and seemed as though it should be penetrated more easily by the digestive juices. Gaulin *et al.* (47) were certain that "Le lait fixe est beaucoup plus digestible." Mayer (83) advised that homogenized milk was more easily digested than nonhomogenized milk and thus was good for sick patients.

Years later, Hiscox (58) stated that homogenization under proper conditions of temperature and pressure operates to reduce the curd tension of the milk, thereby rendering it more readily digestible.

Espe and Dye (45) believed that any factor that tended to make the digesting mass more porous, to lower curd tension or cause it to absorb gastric juice more rapidly, followed by peptonization and disintegration or liquefaction, shortened the digestive period. Working with natural soft-curd milk, they observed that doubling the curd tension of milk increased the length of the digestion period from 30 to 65 per cent.

Anthony (5) noted that curds from homogenized milk were porous and permeable, thus indicating easy admission of the digestants. Lundstedt (80) believed that homogenized milk was to be preferred to ordinary whole milk, not alone because it was more easily digested but because of its better taste. Hull (62), according to Babcock (8), reported digestion studies which showed that milk homogenized at 1,500 and 2,500 lb. pressure at a

temperature of 130° F. gave results almost the same as those obtained from regular milk.

However, Chambers *et al.* (27) concluded from their studies that the curd-surface area was an adequate index of the relative digestibility of milk in the infant's stomach. They demonstrated that some homogenized milk was improved in digestibility by processing to such an extent that it could be fed without further modification to premature and newborn infants.

Babcock (8) believed that the smaller curds of homogenized milk afforded a greater surface area for contact with the digestive juices, which would seem to make the milk more readily digestible than the nonhomogenized milk. McLester (84) stated the digestibility of milk could be enhanced by homogenization.

Speed of digestion of homogenized milk. Doan (36), in a critical review of the literature on soft-curd milk, stated that homogenized milk (including sonized) had not reacted very favorably in some *in vitro* studies, so that until further studies were made and particularly until careful clinical comparisons were available, little could be said in favor of soft curd milk. He (35) explained that homogenized milk did not exhibit as good digestibility characteristics as the curd tension would seem to indicate, apparently because the coagulum, although soft compared with unprocessed milk, was adhesive and held together in a mass instead of breaking up into a granular or flaky condition. Homogenization undoubtedly improved the digestibility of milk but the curd tension apparently was not an index of the improvement.

Later, Doan and Dizikes (38) found that the correlation between curd tension value and digestibility for more than 100 samples of a number of different types of milk, including homogenized, was rather poor. Their observation in part follows:

"While homogenized milk showed curd characteristics and digestion properties much superior to unhomogenized milk, particularly at the second and third hours, it was considerably inferior to acidified, superheated and evaporated milk and appreciably inferior to boiled milk. Sonized milk was slightly inferior to piston homogenized milk, as might be expected, since, in general, sonic vibrated milk is less effectively homogenized than milk treated with a piston machine."

Babcock (7, 8) found in *in vitro* studies that during the first 15 minutes of digestion 76.5 per cent and 56.5 per cent more digestion took place with the boiled and homogenized milks, respectively, than took place in the raw milk from which they were prepared. However, at the end of 2 hours, 15.4 per cent more digestion had taken place in the homogenized milk than in the raw milk, whereas only 10.3 per cent more digestion took place with the boiled milk than with the raw milk. At the end of 5 hours, digestion was practically the same for the raw and processed milk. These results indi-

cated that both boiled milk and homogenized milk were more quickly but not more completely digested than raw milk.

Kelly (73) reported that the relative rate of digestion of properly homogenized milk was similar to that of boiled milk.

Stomach-emptying time. Doan and Welch (41) observed that soft-curd milk appeared to be digested more rapidly and to be eliminated sooner from the stomach of humans, calves and rats than hard-curd milk. Examination of stomach contents revealed that the curd formed was different, the curds from milk of low tension being more friable and looser in makeup than those from milk of high tension.

Chambers and Wolman (26) found that when curd surface areas were compared with the usual curd tension, there was general agreement with the theory that curd tension is an index of the rate of gastric clearance, but exceptions did exist with those milks which were subject to drastic additions or subtractions.

Jeans *et al.* (67) stated that "Milk which produces a soft curd in the stomach leaves the stomach more quickly and is more readily digested than ordinary milk." Tretsen (108) noted that homogenized milk passed through the digestive tract of rats much faster than nonhomogenized milk. Wilcox (125) reported, as a result of roentgenological studies on adults, that homogenized milk left the stomach sooner than soft-curd and average milk.

Hadary *et al.* (50), using roentgenographic examinations of patients who ingested bariumized milks, concluded that no correlation existed between the curd tension of the bariumized milks and stomach or colonic emptying time of children and that soft-curd milks did not leave the digestive tract more rapidly than the hard-curd milks. They observed that at the 2-hour interval "the average per cent of stomach emptiness in the case of homogenized milk did appear to be significantly greater than for the other milks. However, this difference was not evident at four and five hours after feedings, all milks showing substantially similar degrees of stomach emptiness. Similarly, no statistically significant difference in elimination from the system was evident at twenty-four hours after feeding for the five test milks."

Wolman (135) concluded "in 72 'matched pair' experiments no measurable differences could be demonstrated in the intragastric responses to 'soft-curd' homogenized pasteurized milk as compared with the more 'hard-curd' plain pasteurized milk." The mean coagulation times of the ingested milks were 19.2 minutes for homogenized milk and 20.3 minutes for the pasteurized milk. Likewise, the mean emptying times were only one minute apart. The slightly more rapid stomach-emptying time when homogenized milk is ingested probably accounts for many of the statements concerning the improvements of the digestibility of homogenized milk.

Completeness of digestibility of homogenized milk. Ladd (77), in 1915, stated:

"Chevalier demonstrated by chemical analyses that the constituents of homogenized milk are more completely absorbed than those of simple sterilized milk. The more finely divided the food, the greater its accessibility to the digestive fluids, and the greater its assimilation. It is interesting to note also that when rennin is added to homogenized milk, the curd which results is a homogeneous flaky paste, resembling closely the curd of human milk."

Nevens and Shaw (87) from their studies concluded as follows:

"Many references are found in the literature of the 'ease of digestibility' of certain kinds of milk, and the term 'more digestible' is also commonly used. Many of these terms are deduced from the observations of physicians in cases in which they have found that one kind of milk agrees with the patient, while another kind causes more or less digestive disturbance or is unsatisfactory for some reason. There are many statements to the effect that evaporated milk and dried milk are 'more digestible' than fresh raw milk.

"The authors believe that their work helps to clarify the situation which now exists with respect to the term digestibility as applied to milk. Claims that homogenization, evaporation, or drying, or a combination of these factors, makes the protein and fat of milk more completely digestible, lack the support of adequate experimental evidence obtained in actual feeding tests. The author's findings, however, do not preclude the possibility that manufacturing processes such as those just mentioned may affect the time required for the digestion of the protein and fat, or that they may make the milk more readily tolerated by some individuals."

The Council on Foods of the American Medical Association (3), reviewing the literature on the digestibility of soft-curd milk, summarized as follows:

"There is evidence that a variety of milk preparations which yield soft-curds are well tolerated and well utilized by infants, children and older persons. In general, milk that has a low-curd tension as determined by appropriate laboratory methods leaves the stomach more quickly than milk that does not have this property. Such digestion as takes place in the stomach is more quickly accomplished when the curd is soft. The evidence is meager, however, that any soft-curd milk are 'better digested' or more completely digested than ordinary boiled milk."

Kelly (72) observed from some preliminary studies that homogenized milk was not only more rapidly but more completely digested than non-homogenized milk. Later he (74) noted that at the end of 5 hours the amount of proteolysis was practically the same for nonhomogenized and homogenized milks, although at 15 minutes 171 per cent more proteolysis took place with the homogenized milk than with the pasteurized milk. Babcock (8) concluded that boiled milk and homogenized milk were more readily but not more completely digested than raw milk.

Doan and Flora (39), after extensive *in vitro* studies on comparative digestibilities of several milks, concluded that curd tension value did not appear to be a reliable index of digestibility for all types of milk, particularly of homogenized milk. Homogenization lowered curd tension consid-

erably but apparently improved digestibility very slightly, if at all. They believed that curd-particle size apparently would be a more accurate index of the digestibility of milk and its suitability for use by infants than would curd tension. Likewise, Turner (116), using an *in vitro* digestibility test, found that homogenization did not alter appreciably the relative digestibility of cow's milk.

Digestion of homogenized fat. In the early days of milk homogenization, Birk (12) concluded that the reduction of the size of fat globules of cow's milk to that of human milk by homogenization possessed no advantage for well or sick infants over milk not so treated. However, Marriott and Schoenthal (82), in a study of the use of evaporated milk in preparation of infant formulas, predicted "Heating does not alter the chemical character of the fat of milk, but as evaporated milk is subject to a process of homogenization, the fat globules are broken up into much finer particles. Just how great a factor this size of the fat globules may be in rendering the fat more digestible is as yet undetermined. One might expect that the rapidity of digestion of homogenized fat would be greater due to the larger surfaces exposed to lipase action." Dorner and Widmer (42) noted that a number of specialists in pediatrics claimed that homogenized milk was dangerous for babies because it formed too much fatty acid in the intestine. Homogenization does accelerate the breaking down of the fat, but the question concerning the danger to infants has not been proved and Nevens and Shaw (87) pointed out that it is often claimed that manufacturing processes, such as homogenization, which reduce the fat globules to smaller size, increase the digestibility of the fat. However, results of their studies raised a question regarding the soundness of such claims, since the digestibility of the fat of fresh whole milk was so nearly complete that there was but little possibility of its being increased.

Likewise, Holt *et al.* (60) showed that the size of the "fat particles is without influence on fat absorption." Also, Marriott and Jeans (81) concluded that the breaking up of the fat globules in itself was of little importance in fat digestion.

V. MISCELLANEOUS FACTORS

Lack of distress from drinking homogenized milk. Brennemann (17) demonstrated experimentally with humans that "the casein of raw milk unless modified so that it will not form hard large coagula offers serious difficulties in digestion that are not present in boiled milk" (soft-curd).

Anthony (5) reported that the regurgitation subjects employed in his study noted a lack of distress from drinking soft-curd milk. Jeans (65) believed that the soft-curd character produced in milk by proper homogenization might be expected to lead to greater speed of digestion of milk and, thereby, possibly contribute to an earlier feeling of hunger and also,

possibly, to a relief from a feeling of overfullness that some adults seem to have as a result of drinking milk.

Spur and Wolman (99) successfully raised more than 200 normal infants on homogenized milk without the children exhibiting symptoms or signs of digestive disturbances attributable to imperfect utilization of the milk within the gastrointestinal tract. Sonic, low-pressure and high-pressure homogenized milks were used in their studies.

The role of homogenized milk during febrile illnesses. Milk usually is included in the dietary of patients during sickness. Some illness involving fever may alter the digestibility of milk since the acid of gastric juice decreases temporarily during fever (15). Wolman (134) discussed this possibility as follows:

"The intragastric milk clotting then becomes altered; the coagulation becomes affected by the pepsin enzyme which, when acting alone, tends to produce large calcium-rich curds. Facilitating this trend toward larger curds is the element of fever itself. As the environmental temperature rises above normal body temperature caseinous clots exhibit a marked tendency to shrink by syneresis and grow tough and rubbery. Thus can be explained the nausea and vomiting occasionally seen following the taking of pasteurized milk during febrile illnesses. This tendency, of course, is less marked with homogenized and other more soft-curd milks, which give rise to smaller, softer, and presumably more readily digested curds."

Calcium, phosphorus and nitrogen retention. Hess, according to Anthony (5), has indicated that reduction of the curd tension is a factor in inducing calcium retention, since it increased the availability of the vitamin D and the minerals in the milk itself by reducing the periphery and toughness of the curds. However, Jeans *et al.* (68), in feeding infants milks which permitted production of fine curds in the infant's stomach (acidified, pepsin-rennin and reconstituted evaporated but not homogenized milk), found: (a) normal growth rate, both in length and weight; (b) normal development and calcification of bone; and (c) a steady increase of retention of the elements studied, all of which constituted evidence that all of these milk mixtures were good for infants. However, in these studies a soft-curd character was obtained by means other than homogenization.

Homogenized certified milk. Elias (44) concluded that soft-curd milk had no decided advantages over other certified milk in digestibility and that it had no unusual tendency to make infants gain weight. On the other hand, Corbin (30) reported favorable results on the use of natural soft-curd certified milk in infant feeding, citing 11 cases of infants ranging from 2 months to 3.5 years.

Wolman (128, 129) believed that physicians would have complete confidence in the soft-curd nature of special homogenized certified milk which could be prescribed for dietary purposes, since certified milk would guarantee to the consumer that the product was all that it was represented to be, not adulterated with reconstituted milk or deficient in butterfat.

Effect on appetite when homogenized milk is taken between meals. A thesis presented before the American Pediatric Society in 1940 indicated that milk required 3 to 3.5 hours for complete gastric digestion and that routine feeding of milk midway between meals was not advisable (134). In extensive studies, Wolman (134) observed that before-meal feedings of nonhomogenized or homogenized milk failed to elicit in any child any undesirable symptoms of anorexia, gastrointestinal distress, or decreased consumption of food. For nearly 5 months, 59 convalescent children (3 to 14 years of age) were given a 7-ounce glass of milk twice daily, 1 hour before meals, homogenized milk (mean curd tension, 10.7 g.) and pasteurized milk (mean curd tension, 33.6 g.) being served in alternate months. Concerning the reported unfavorable experiences sometimes encountered with milk before meals, he comments, in part, as follows:

“One hypothesis for the recounted unfavorable experiences with milk before meals suggests itself. In extremely cold weather and under special circumstances, market milk sometimes develops a high content of casein and fat and grows markedly hard-curd. Such milk—of curd tension often over 50 gm.—may give rise to tough rubbery clots inside the stomach. Occasionally a child may suffer from a temporary reflex loss of appetite as a result.”

A later report by Wolman (135) on the physiology of milk digestion during childhood showed that following the drinking of 8 ounces of milk the gastric-emptying time ranged from 50 to 170 minutes, the mean of 122 experiments being almost exactly 120 minutes. No appetite-impairing effect from milk occurred.

The effect of drinking milk between meals on the quantity of food taken spontaneously at mealtimes. Wolman (134) subjected 18 children to a quantitative 3-week diet-intake study in which extra milk, pasteurized and homogenized, was given in 7-ounce quantities 1 hour before each meal for 2 of the 3 weeks, and the amount of food taken spontaneously during mealtimes was measured carefully. He found that the feeding of either type of milk before meals had no effect on the amount of food consumed at mealtimes. Thus, the data indicated that extra servings of milk might improve the child's nutritional status or food intake, and homogenized milk was neither superior nor inferior in this respect.

Increased consumption. Irwin (63) reported that after the introduction of homogenized milk at the Mont Alto Sanitarium in Pennsylvania, the consumption of milk increased from 1.5 to 2 quarts of milk per capita per day. Likewise, Hollingsworth (59) reported an increase in milk consumption. He stated, “There is no doubt but that homogenization of milk has increased the per capita consumption of milk and milk products. A pleasing glass of milk of uniform quality and richness has a tendency to enhance, everyone will admit, the consumption of that product. Homogenization has brought about increased consumption of milk products.”

Wolman (134) believed that the improved nutritional quality as a result of the homogenization process would be reflected in an increased volume of consumption.

VI. HOMOGENIZED MILK IN INFANT FEEDING

Introduction of and emphasis on soft curd. Many of the observations and researches, especially the earlier ones, on the use of cow's milk in infant feeding centered around soft-curd milk. Although some of the earlier studies had little to do with homogenized milk, they are included herein in order to present a more complete picture of the role of homogenized milk in infant feeding. An exception is the early observation of Variot (117) who, noting the soft, diffuent, creamy curd formed from homogenized milk, successfully administered homogenized milk to a hundred nursing babies of various ages, some having more or less gastro-intestinal disorders. However, it was in the feeding of newborn and debilitated infants afflicted with gastro-intestinal disturbances that homogenized milk was used most successfully. He observed, "Generally at the end of a few days, vomiting ceased, the stools acquired normal consistency and color, the growth in weight followed its normal course." Mention should be made, however, that homogenized milk at that time was sterilized and, except for concentration, likely was more similar to evaporated milk than to present day homogenized milk.

Apparently a quarter of a century was to pass before there was any renewed interest in the use of homogenized milk in infant feeding. Meanwhile, many studies were made on soft-curd milk.

Brennemann (17) demonstrated experimentally, using humans, that boiled, soft-curd cow's milk did not form large, hard coagula in the stomach as did the raw milk. As a result of extensive studies on the coagulation of cow's milk in many modifications in the human stomach, he (18) emphasized that "*cow's milk is not a liquid food, but a solid food—so solid, in fact, that in babies the curds found in the stomach often pass through the whole intestinal tract and appear in the stools as large, hard, beanlike curds.*" He noted in a series of human regurgitation studies that raw milk was a very solid food and that boiled milk was a semi-liquid food. The solidity of the curd could be modified by many methods. Dried and condensed milks, as a rule, formed a minimum curd. Later, he (19) summarized: "It is known that cow's milk is a peculiarly solid food; that it coagulates in large firm masses in the stomach; that these coagula become larger for about two hours by agglutination and continuously and increasingly harder by contraction and that all digestion is at the periphery only. This is as true of boiled as of raw milk, of diluted as of whole milk, except that in each former case all of the changes are less marked. Nowhere else would one venture the opinion that the size and consistency of the ingested

bolus, and especially of such a bolus, would not be an important factor in digestion."

Buckley (20) believed that the inability of infants to digest and assimilate raw untreated milk from some cows and to assimilate similar milk from other cows was due to the curd character of the milk.

Dennett (31) concluded from clinical studies that boiled milk aided in overcoming digestive disturbances in infant feeding and that it did not cause digestive disturbances in normal infants.

Hill (54) early pointed out that the curd tension of milk might serve as a guide in the selection of milk for infant feeding and (55) that the physical curd character, as determined by the curd test, was an index to the comparative digestibility of the milk by delicate infants. Later, in discussing the nutritional advantages of natural soft-curd milk, he (56) stated, "If it is possible by supplying soft-curd milk to obviate the necessity of using a formula and to allow the use of the milk as it comes from the bottle, a great service will have been rendered to mankind. Soft-curd milk is not confined to infant nutrition alone. In case of adult indigestion and in gastric ulcers it has been used with remarkable results. It can thus become a boon to invalids and mature persons in general as well as to infants." Summarizing a decade and a half of soft-curd studies, he (57) reported that feeding tests with soft-curd milk conducted in different sections of the United States have been favorable to the use of soft-curd milk. He stated that when infants are fed a natural unmodified milk they do not acquire a dislike for milk as they grow older, as often is the case when they are fed a sweetened modified milk.

Barnes (9), with limited observations of babies fed on soft-curd milk, stated that the results seemed to show that this type of milk feeding was superior to hard-curd milk and that quite possibly many difficult feeding problems could be solved by using it.

Scales (91) stated that soft-curd milk formed a soft, flaky mass in the stomach in contrast to the coherent, rubbery mass of hard-curd milk which caused regurgitation and indigestion to the infant.

Elias (44) concluded from his observations on feeding 82 babies for periods from 10 to 90 days on soft-curd milk (natural soft-curd, boiled certified and evaporated) that soft-curd milk had no decided advantages over other certified milks in digestibility and that it had no unusual tendency to make infants gain weight. Neither did it have any special value in preventing or treating vomiting and diarrhea.

Doan (33), summarizing the advantages of natural soft-curd milk, believed that since babies having difficulty with ordinary milk, showing no gain in weight and troubles with regurgitation, almost without exception ceased vomiting and began to gain as soon as soft-curd milk was used; and since patients in hospitals and sanitoriums were usually subnormal and

many were afflicted with poor digestion, that soft-curd milk would be demanded in the future for these groups of consumers.

Based upon studies on a group of 60 infants ranging from birth to 6 months of age fed on soft-curd milk for a period of time ranging from 2 to 8 weeks (44 were fed over a 7-week period), Morris and Richardson (85) observed that normal milk of low curd-tension was low in energy value and when infants were fed to satiety they needed more normal soft-curd milk than ordinary milk. Thus, they concluded: "Considering (a) the variability in the production of soft-curd milk and the increased observation necessary in its production; (b) the lack of superior results when used in comparison with other accepted infant formulas, soft-curd milk does not warrant special production and certification for use in infant feeding."

The Committee of the American Dairy Science Association (1) on methods of determining the curd tension of milk advised that the curd tension value of milk should not be considered an absolute index of its digestibility or of its suitability for infant feeding purposes. They believed, however, that the values did correlate in a general way with those properties and, thus, the determination appeared to be the best simple method for the purpose available.

Marriott and Jeans (81) stated: "Soft-curd milks are advantageous in that they produce a softer, finer curd in the stomach, and therefore leave the stomach more quickly and are more quickly digested. This advantage, however, is largely for the person past infancy, since all milk for infant feeding should be boiled, and boiling is effective in reducing curd-tension sufficiently for the milk to have soft-curd properties."

Introduction and use of homogenized milk for infants. Washburn and Jones (121), noting that curds from homogenized milk were so much more flocculent and friable than those from nonhomogenized milk, believed that nutritional benefits might be expected from such treatment.

Eichholz (43) recommended homogenized milk for infants and for forced feedings. However, the milk used by him, in addition to being homogenized, was sterilized, which would reduce further the curd tension.

Clay (28) reported that homogenized milk gave good clinical results in cases of difficult feeding. He believed that if a milk of low specific gravity and low curd tension were homogenized, with the resultant subdivision of the fat globules and the reduction of the curd tension to 10 g. or lower, a raw milk very suitable for infant feeding would result. (Danger of rancidity resulting from homogenization of raw milk was not widely known at that time.)

Berry (11), in two feeding trials with rats, noted in both trials that the rats fed normal hard-curd milk which was homogenized to produce a soft curd made the largest average gains.

Wolman (127) stated, "These new products (soft curd and low tension

milks) break down the last obstructing fortress on the road to the perfect digestion of milk and represent an important contribution to the healthful feeding of infants, children and sick adults. Improved nutritional quality will be reflected undoubtedly in an increased volume of consumption." Later he (130, 131) concluded that pasteurized homogenized milks used in their studies were found to be "as good or better as a food for infants than pasteurized milk boiled for five minutes in the home. Such mechanically processed milks may be safely fed to infants and young children; in fact the danger of accidental household contamination is less than when unprocessed milk is employed." However, he (131) felt that over the country as a whole the status of homogenized milk was not yet at such a uniformly high peak that indiscriminate feeding of normal babies without boiling of the milk could be considered safe. Nevertheless, the fact cannot be ignored that homogenized milk must be a pasteurized product (22, 42, 51) and that the addition of raw milk invariably causes a rancid flavor (42, 49, 79), thus adversely affecting its palatability and reducing its consumption.

Later, Wolman *et al.* (136) compared homogenized milk (sonic, low pressure, and high pressure) with boiled, pasteurized milk as a base for infant formulas in feeding trials with over 200 infants for 2 to 12 months. They observed a low incidence of constipation, diarrhea, vomiting and kindred gastro-intestinal upsets in all groups; in digestibility and safety, the homogenized milks proved as satisfactory as the control boiled milk.

Wilson (126) concluded that soft-curd milk seemed to have special value for infant feeding and for some older children and adults, but he questioned if one method of producing soft-curd milk was superior in all respects to any other method.

Kugelmass (76) noted that the tolerance of infants for milk has been enhanced by homogenization, which softened the curd by increasing the amount of casein adsorbed on the surface of the fat particles.

Jeans (66) believed that from the point of view of a pediatrician, homogenized milk had two advantages: the vitamin D of fortified homogenized milk went to those who drank the milk, and it was more rapidly digested because of the effect on the casein curd. However, he believed that most pediatricians would not consider homogenization important in infant feeding because milk formulas should always be boiled for bacterial safety, but, after the formula period, homogenization is useful in making milk more readily digestible.

Homogenized milk in the preparation of infant formula. The use of homogenized milk in the preparation of infant formulas seems to have found favor both with the mothers and with the physicians. Clay (28) advised that homogenization made it possible to use a soft-curd milk without having to boil the milk.

Wolman (127) stated that "homogenized milk makes an excellent foundation for infant formulas. . . . The curds obtained are always much finer than the curds of formula derived from identical specimens of milk pasteurized but not homogenized." Later (131) he noted that the making of formulas with homogenized milks was markedly simplified, stating: "Elimination of the steps of boiling, filtering and cooling saved the nurses and mothers an appreciable number of minutes each day, and reduced the opportunities for accidental household contamination of the contents of the formula bottles." Furthermore, making a formula with the cold milk (homogenized) was simpler and better than heating and allowing the prepared formula to cool over an extended period, thus permitting bacterial growth (136). By use of holding pasteurization temperatures from 150 to 160° F. for 30 minutes, the bacterial counts of homogenized milks could be kept at low levels, usually below 2,000 per ml., resulting in high sanitary quality and eliminating introduction of an objectionable heated flavor (136).

Blatt (14), following an 18-month study, concluded that commercial pasteurized milk with a curd tension reduced to below 20 g. by proteolytic enzyme action was desirable for infant feeding, since it was well tolerated and digested and, in addition, was easily, economically and safely prepared. He believed also that in its preparation the bacteriostatic value of handling a food product at a low temperature was utilized in contrast to the practice of making the milk soft curd by boiling and allowing it to cool slowly.

Glynn (48), commenting on the observations of Conquest *et al.* (29), who found that low curd tension milk yielded a soft curd which passed through the stomachs of calves more rapidly than normal curd tension milk, stated that successful feeding for each species must employ a milk of curd tension physiologically adequate for the gastric digestive apparatus of that species. Thus, he believed milk for successful infant feeding should have a soft-curd character.

Espe and Dye (45) pointed out that normal milk of high curd tension usually meant a more concentrated milk than one of low curd tension and, therefore, one of greater food value. Thus, a hard-curd milk rendered soft by homogenization would seem to have a greater caloric value than a natural soft-curd milk.

DISCUSSION

The general acceptance of homogenized milk would indicate that the nutritive value of the milk is not impaired. On the other hand, the possible enhancement of the nutritive value of milk by homogenization would seem to depend upon the importance in nutrition of the roles of such factors as palatability, homogeneity, satiability, digestibility, and retention and utilization of its constituents.

Probably the chief value of homogenization of milk from the nutritive standpoint is the fact that the process renders the milk homogeneous throughout. There can be no pouring off of the cream layer, leaving the partially defatted milk for those less fortunate. However, should there be no need for butterfat calories, this property becomes a distinct disadvantage. Properly homogenized milk always will carry its full caloric value because the fat cannot be removed by pouring. Associated with the milk fat are the vitamins A and D. Those who get the fat get these vitamins. Homogenized milk is better suited for vitamin D fortification than is nonhomogenized milk, for the added vitamin D associated with the fat will remain uniformly distributed in such milk. Children drinking vitamin D-fortified homogenized milk will be assured of their portion of vitamin D, an important factor in nutrition.

A second important property of homogenized milk is its palatability. Homogenization fixes the fresh flavor of milk so that the fat of milk does not oxidize readily, yielding the old, stale, cardboard, oxidized flavor which is particularly objectionable to children. Not only does the process fix the flavor of milk at time of homogenization, but it stabilizes the fat of adequately processed and properly stored homogenized milk over a long period of time. In every-other-day delivery, this is especially important. When the milk is several days old, it is as palatable as when first produced. Also, there are no thick cream plugs, flecks or butter granules floating on a glass of homogenized milk. To children these seemingly foreign particles are particularly nauseating and objectionable. Casual reports and general observation, not based on scientific evidence, indicate that children take to homogenized milk because it is sweet, smooth and uniform in consistency. Generally, there is less of a milk-drinking problem when children have access to homogenized milk.

Homogeneity and palatability would seem to be major advantages of homogenized milk effecting greater consumption of milk and, therefore, improving human diets. However, these qualities are often overlooked or overshadowed by the claims of "greater digestibility", "more digestible", and so on, as a result of the softening of the curd by proper homogenization. Claims that homogenization makes the protein and fat of milk more completely digestible seem to lack the support of adequate experimental evidence obtained in controlled feeding trials. Nevertheless, data indicate a slightly faster stomach-emptying time when homogenized milk was ingested than when nonhomogenized milk was taken into the body. The desirability of rapid stomach-emptying time is sometimes questioned, some believing that homogenized milk does not "stick to the ribs" and hunger soon manifests itself. However, rapid stomach-emptying time would seem to be an advantage, especially in infant feeding. Associated with rapid stomach-emptying time seems to be a lack of distress after ingesting homogenized

milk. That homogenized milk plays a real role in infant feeding is supported by observations made by authoritative pediatricians.

SUMMARY

An attempt has been made to make this review as complete and unbiased as possible. To this end opinions, observations and data bearing on the subject have been gleaned from the literature. That many data were conflicting is a foregone conclusion. Nevertheless, the majority of the data reported herein would seem to support the following conclusions concerning homogenized milk:

1. The homogeneity of properly processed homogenized milk assures equal distribution of the fat, vitamin A, and added vitamin D to those who consume the milk.

2. Homogenization retards or inhibits the development of the copper-induced oxidized flavor over an extended period of time. Thus, the milk properly stored and refrigerated retains the fresh flavor which contributes much to its palatability.

3. The smooth, uniform consistency of properly homogenized milk, as shown by the absence of cream flecks, non-miscible cream or butter granules, further contributes to its palatability.

4. Proper homogenization changes normal hard-curd milk to a soft-curd milk, which appears to be digested slightly more easily but neither more quickly nor more completely than hard-curd milk despite a faster stomach-emptying time.

5. Reduction in the size of the fat globules by homogenization does not increase the digestibility of the fat, since the digestibility of the fat of fresh whole milk is so nearly complete that any marked increase in digestibility is not possible.

6. Homogenized milk has been used successfully in the preparation of infant formula and in infant feeding.

7. The ingestion of homogenized milk seems to be associated with a lack of distress and a sensation of overfullness during digestion. Hence, homogenized milk would seem to be especially suited for hospital dietaries.

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ABSTRACTS OF PAPERS PRESENTED AT THE FORTY-THIRD ANNUAL MEETING

PRODUCTION SECTION

- P1 The Relative Merits of a Cow's Own Record and Her Progeny Test for Predicting the Butterfat Production of Her Future Daughters. W. J. TYLER AND GEORGE HYATT, JR., West Virginia University.

Wide differences of opinion now exist concerning the relative importance of a cow's production records and her progeny test in estimating a cow's breeding value or transmitting ability. The objective of this study in dairy cattle breeding was to compare the relative value of one unselected record of a cow and her daughter's records in estimating butterfat production of future daughters.

Data consisting of first records on 1,249 Ayrshire cows and their daughters were analyzed to determine the relationship between one unselected record of a cow and the production of her daughter. Also, correlation coefficients between the production records of one, two or three daughters and the records of additional daughters were calculated. The results indicate that one unselected record of a cow may be as important in predicting the production of her daughters as the average of the records of two or three daughters are in estimating the butterfat production of additional daughters. The intra-herd correlation coefficients varied from 0.10 to 0.26, while the gross correlations were from 0.18 to 0.38. A combination of the record of the cow and a record for one or two daughters was better than either one alone in predicting the production of future daughters.

- P2 Preliminary Results from the Crossing of Two Inbred Lines of Holsteins on Growth and Milk Production. N. P. RALSTON, S. W. MEAD, AND W. M. REGAN, University of California.

The University of California Holstein herd was inbred for 11 years through sire-daughter matings. Females with a relatively high coefficient of inbreeding (Wright's— F) were smaller at birth, had a higher mortality rate, and developed into smaller mature animals than the outcross generation. Butterfat production decreased with each successive generation of sire-daughter mating until females with an F of 37.5 and above produced 199 lb. less than the first-generation daughters or 206 lb. less than the foundation females.

An unrelated sire ($F = 23$) mated to the first sire's inbred daughters (average $F = 29.8$) produced offspring averaging 13.4 lb. heavier at birth than their dams and 6.2 lb. heavier than the foundation cows. Such off-

spring also made more rapid growth and attained a greater ultimate size. Butterfat production of these outcross cows was 203 lb. more than for their dams and 52 lb. greater than for the foundation cows. This sire is being mated to his daughters in an attempt to determine the reason for the increase in growth and butterfat production. Thus far, birth weights and body measurements indicate that heterosis is involved.

P3 Genetic Variation in the Levels of Blood Plasma Carotene and Vitamin A in Dairy Cattle. R. E. MATHER, University of Wisconsin.

Blood samples were collected from calves, heifers, and cows from one Holstein and two Guernsey herds at intervals averaging 5.5 weeks from June until August of the following year. The plasma was analyzed for total carotenoids and vitamin A. The relationships of carotene and vitamin A with season, age of the animals, level of milk production and length of period in milk were studied. Appropriate corrections then were made and the heritability within season was determined by correlations between related animals.

Combining the correlations for the different age and breed groups through the "z" conversion and including two negative values gave an average correlation between carotene levels of daughter and dam of 0.1324, which was significant for 230 degrees of freedom. For vitamin A the average correlation was 0.1555, which also was significant. Doubling these values gave "heritabilities" of 0.26 for carotene and 0.31 for vitamin A.

P4 Measurement of the Rate of Endocrine Gland Secretion as a Tool in the Genetic Selection of Dairy Cattle. C. W. TURNER, Missouri Agricultural Experiment Station.

Genetic improvement in the "capacity for milk and fat production" of dairy cattle has been retarded seriously due to the lack of measures of the several factors which combine to make possible high production. Since it now has been demonstrated that the growth of the udder, the initiation and maintenance of milk secretion, and the "let down" process are controlled by the hormones secreted by the various endocrine glands, it is obvious that the rate of secretion of the several hormones is the key to the problem.

The problem can be broken down into the following component parts:

<i>Hormones</i>	<i>Controls</i>
1. Estrogen	Act upon anterior pituitary
2. Progesterone	to secrete mammogen.
	Udder size, amount of secreting tissue.

¹Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 1110.

- | | | |
|------------------------------|---|--------------------------------|
| 3. Lactogenic hormone | Acts upon udder cells to secrete milk. | Persistency of milk secretion. |
| 4. Other pituitary hormones | Stimulate the production of the precursors of milk. | Secretion of milk. |
| 5. Thyroxine | Acts upon blood supply. | Increases secretion of milk. |
| 6. Parathyroid hormone | Regulates the Ca and P of the blood. | |
| 7. Adrenal cortical hormones | Mode of action not clear. | Essential for lactation. |
| 8. Pituitrin | Acts upon the "let down" of milk at milking time. | |

When all of the hormones involved in these processes are being secreted in optimum amounts, one might expect high milk production. The secretion of any one hormone in limited amounts will limit the capacity of the animal. Methods of detection of the limiting hormones will be explained.

P5 Thyroid Secretion Rate and Its Relation to Various Physiological Processes.¹ VICTOR HURST AND C. W. TURNER, University of Missouri.

The knowledge of the thyroid secretion rate in an animal aids in understanding the relationship of the thyroid gland to various physiological functions such as growth, reproduction and lactation. In the growing mouse, the administration of thiouracil retards growth, but animals maintained normal growth when receiving thiouracil plus thyroxine injected in amounts approximating the normal thyroid secretion rate. Injecting thyroxine in larger amounts stimulated growth as long as physiological dosages were administered.

The thyroid secretion rate per 100 g. body weight declined during the growth period, and there were indications that the reduction of thyroxine dosage during a rapid period of growth coinciding with a declining thyroid secretion rate stimulated growth to a greater extent than did the administration of a constant dosage of thyroxine over the same period. Thyroid secretion rate varied among mature mice according to strain, and within a given strain there were sex differences in thyroid secretion rate. Castration decreased and the feeding of dianisylhexene (dimethyl ether of diethylstilbestrol) increased the thyroid secretion rate.

Evidence is presented to show that thyroxine does not pass through the mammary gland of the mouse into the milk. Lowering the environmental temperature below the zone of thermoneutrality in the mouse increased the thyroid secretion rate in both males and females. Approximately 7 per cent of the thyroxine in thyroprotein was utilized by mice when it was administered orally, as compared to subcutaneous injection.

¹ Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 1105.

These studies emphasize the importance of using the normal thyroid secretion rate of animals as a reference base in studying the effects of hypo- and hyperthyroidism upon the various physiological processes of the animal body.

P6 The Effect of Low Levels of Thyroprotein Feeding upon Milk and Milk Fat Production, Body Weight, Body Temperature, Heart Rate and Respiration Rate of Dairy Cows. R. G. SWANSON AND C. B. KNOTT, The Pennsylvania State College.

Two trials have been conducted to determine the effects of feeding daily 0.625, 1.25 and 5.0 g. of thyroprotein for 1 year to dairy cows. In the first trial, 12 Holstein cows were divided into four comparable groups. These groups were assigned at random to the several levels of thyroprotein feeding, as well as to a control group. The T.D.N. intake of the cows was 125 per cent of the Morrison Standards for good cows under usual conditions. On the basis of 4 per cent F.C.M., M.E., there was no significant difference in milk production between the thyroprotein-fed groups and the control group. No differences were found between the groups in body temperature, heart rate, respiration rate, gain in body weight or breeding efficiency.

The second trial was conducted with 32 cows divided into four comparable groups. In this trial Ayrshire, Brown Swiss and Guernsey cows were used. Their T.D.N. intake was 110 per cent of Morrison's Standards for good cows under usual conditions. Thyroprotein was fed at the same levels as in the first trial for 1 year, including the dry period. All groups gained in body weight and had similar body temperatures, heart rates, respiration rates and breeding efficiency. At this level of T.D.N. intake (110 per cent), thyroprotein appeared to stimulate milk production.

P7 Effects of Feeding Thyroprotein to Milking Cows in Summer. K. E. GARDNER AND T. W. MILLEN, University of Illinois.

In an experiment run during the hot summer weather of 1947, 12 cows receiving thyroprotein¹ at the rate of 1.33, 1.5 and 2.0 g. daily per 100 lb. body weight showed fat yield increases of 32, 48 and 50 per cent, respectively. During July and August, the cows fed thyroprotein lost weight rapidly, although receiving 140 per cent of Morrison's minimum T.D.N. requirement. When the weather cooled, large weight gains occurred on intakes above 130 per cent of requirements.

Body temperatures of Holsteins receiving thyroprotein averaged 105° F. when the environmental temperature reached 96° F. and averaged 1.5° F. higher for the trial. Cows on the highest rate of thyroprotein exceeded respiratory rates of 100 per minute on the hottest day and averaged

¹ The thyroprotein used was "Protamone", furnished by the Cerophyl Laboratories, Inc., Kansas City, Missouri.

78 compared to 49 for controls. The pulse rates averaged 95 compared to 49 for controls. There were no outstanding differences in blood pressure as measured using the broad cuff on the tail (Harshbarger, University of Illinois, unpublished). Values for hemoglobin, serum calcium and inorganic serum phosphorus showed no trends attributable to the thyroprotein feeding. All cows, including controls, showed a marked lowering of serum calcium and magnesium in midsummer and a rise in September.

P8 Effects of Feeding Thyroprotein during Successive Lactation. J. W. THOMAS AND L. A. MOORE, Bureau of Dairy Industry, U. S. Department of Agriculture.

Thyroprotein has been fed to 12 dairy cows for successive lactations, beginning at 50 days postpartum and continuing until 90 days before the next parturition. All the cows were normal at parturition time and all the calves appeared normal.

Milk production during the first 50 days of successive lactations was compared. During this segment of the second and third lactations, the cows fed normally produced 132 per cent as much milk as they produced during their first lactation. Cows that had been fed thyroprotein for one or two lactations produced only an average of 117 per cent during the first 50 days in their second and third lactations as compared to the first lactation.

The average butterfat test during the first 50 days of the second and third lactations for 13 normally fed cows was 106 per cent of the value for the first lactation. A corresponding value of only 89 per cent was found for eight cows that had been fed thyroprotein during their first and second lactations. In the second and third lactations following thyroprotein administration, an increase in milk production and butterfat percentage always was observed. However, the total production during the second and third lactations was not much greater than that observed during the first lactation.

Production data and plasma protein-bound (thyroxine) iodine values indicate that the thyroid gland may be functioning at a subnormal rate for at least 140 days after the cows have received thyroprotein for one or two lactations.

P9 Factors Controlling the Extent of Duct Growth in Mammary Glands.
I. The Influence of an Estrogen in a Hereford Heifer. RALPH P. REECE, New Jersey Agricultural Experiment Station.

Of two sexually immature Hereford heifers, one was injected subcutaneously once weekly for 16 weeks with 5 mg. of estradiol dipropionate.

Six days after the initial injection the experimental heifer was in estrus; however, a rectal palpation of the ovaries indicated that they were immature. Estrus also was observed in the control heifer during the experiment. At the time of slaughter, the udder, teats, and pituitary gland of the injected heifer were larger than those of the control heifer. Histological preparations showed a greater hyperplasia and hypertrophy of the glandular tissue near the gland cistern in the injected heifer than in the control heifer. The distance that the duct system extended from the base of the teats was essentially the same in the two heifers. Ovulation had occurred in the control heifer but not in the injected heifer.

P10 The Value of Oxytocin for Reducing Fluctuation in Milk and Fat Yield during Experimental Periods. H. P. ADAMS AND N. N. ALLEN, University of Wisconsin.

The daily variations in milk and fat production and in fat percentage confuse the measurement of small differences in these values resulting from experimental treatment. These variations are due in part to incomplete removal of the milk from the mammary gland.

This experiment, using eight cows, was conducted to determine whether the use of oxytocin to insure complete milking will reduce materially the daily variations. A reversal plan was followed with 10-day experimental periods. Under normal feeding, oxytocin administered prior to milking did not reduce significantly the variability in milk or fat yield or fat percentage, as compared to a good milking routine without oxytocin. Comparing high and low fat intakes, the effect of the high fat intake on fat production and test was shown as clearly without as with oxytocin. While the oxytocin did not reduce variability, it did cause a highly significant increase in milk and fat yield, with no significant effect on fat percentage.

In order to secure information as to why the extra milk, presumably residual milk of high fat content, did not increase the test, cows were milked in three approximately equal portions, which were tested separately. Under normal milking, the first portion had the lowest and the last portion the highest fat content.

During a second period, oxytocin was injected following the regular milking to secure any residual milk remaining. After the first oxytocin milking, it was found that the amount of milk in the third portion before injection was reduced. The residual milk secured following injection was higher in fat than the third portion, but the first portion of the succeeding milking was abnormally low in fat content, offsetting the effect of the high fat, residual milk. This is interpreted as indicating that the residual milk under ordinary milking practices is secured in the normal milk of the following milking.

P11 The Role of Certain Hormones in Spermatogenesis.¹ J. D. SAMPATH KUMARAN, University of Missouri.

The investigations reported deal with the effects of various hormones on growth and development of the testes and spermatogenesis. The experimental animals were White Plymouth Rock cockerels. In these birds, the appearance of the first primary spermatocyte was at 42 days, the secondary spermatocytes on the seventieth day, and spermatids on the eighty-fourth day after hatching. During the winter this process was delayed about a week. Through the use of the polarizing microscope, birefringence was observed in the region of the cells of Leydig. Since the steroid hormones give this reaction, it has been interpreted as indicating that the male hormone is secreted by these cells.

Hypothyroidism, induced by feeding 0.3 per cent thiouracil in the ration, started on the 12th week and continued for 30 days, inducing regression of testis size and spermatogenesis. When testosterone at the rate of 20 mg. per kg. feed was fed with 0.3 per cent thiouracil, testis size was brought back to normal. Thus thiouracil does not possess toxic effects at this level of administration which cannot be corrected by the thyroid hormone; this hormone in amounts above normal actually stimulates growth of the testes and spermatogenesis. The feeding of the dimethyl ether of diethylstilbestrol at the rate of 4 mg. per 100 lb. feed to the same type of birds caused regression of the testes to one-tenth the normal size. The feeding of 10 per cent dried cow manure did not correct this condition.

P12 The Relationship between Type Ratings of Ayrshire Females as Young Heifers and as Cows. GEORGE HYATT, JR., AND W. J. TYLER, West Virginia University.

Since type is heritable and important to breeders of dairy cattle, it is very desirable that dairymen be able to determine which are the poorest type animals in their herds at as early a date as possible. A cooperative project was inaugurated in 1942 by the West Virginia Agricultural Experiment Station and the Ayrshire Breeders' Association to determine the repeatability of heifer classifications at 6-month intervals and the correlation between the several classifications of a heifer and her classification after freshening.

One hundred and two Ayrshire heifers, each of which has been classified at least once following freshening, have been rated for type each 6 months during the period from October, 1942, through April, 1948. The inspectors classified each heifer into one of five groups (Excellent, average score 90-100; Very Good, 85-90; Good Plus, 80-85; Good, 75-80; Fair,

¹ Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 1107.

70-75), similar to the classification program of adult animals. When these 102 animals were placed in four groups (Group I, below 80.5; group II, 80.5-82.5; Group III, 82.6-85.0; and Group IV, above 85.1) according to the averages of their several ratings before calving and when these averages were compared with the group average after freshening, the following results were obtained: Fourteen animals in Group I averaged 78.6 before freshening and 80.1 after freshening; 34 in Group II, 82.0 before freshening and 81.5 after freshening; 31 in Group III, 84.4 before freshening and 83.0 following freshening; and 23 in Group IV, 87.1 before freshening and 84.5 after freshening. The correlation between the several ratings of the heifers before calving with their ratings after calving was approximately 0.35, which is only slightly lower than the correlation between the consecutive ratings of mature cows when rated by different judges.

P13 Effect on the Blood Vitamin A Activity Values for Dairy Animals of Certain Vitamins and Minerals. DWIGHT ESPE, D. W. BOLIN, AND F. M. BOLIN, North Dakota Agricultural College.

Thirty dairy cows were fed for 18 weeks a ration of prairie hay, corn silage, grain, a carotene concentrate (equivalent to 90 mg. of carotene daily), and certain vitamins or minerals. At the rates fed, cobalt, iodine, or *alpha*-tocopherol had no influence on the carotene and vitamin A values of the blood of these cows. The adding of a small amount of vegetable oil to the carotene concentrate did not alter the carotene and vitamin A values occurring in the blood.

Twelve heifers were fed prairie hay, grain and a carotene concentrate or dehydrated alfalfa. Forty-five mg. of carotene daily in the carotene concentrate or dehydrated alfalfa hay which was fed exerted a negligible effect on blood carotene and vitamin A values.

P14 Effect of Certain Soybean Products on the Concentrations of Carotene and Vitamin A in the Milk Fat and in the Blood Plasma of Dairy Cows. R. L. SQUIBB, C. Y. CANNON, AND R. S. ALLEN, Iowa State College.

Two trials were conducted using paired Holstein-Friesian cows to determine the effect of raw soybeans and soybean oil in the first trial and the effect of raw soybeans and soybean oil meal in the second trial on the concentration of carotene and vitamin A in blood plasma and milk fat. Feeding raw soybeans in amounts of 9 lb. daily per lactating cow caused the blood plasma concentrations of carotene in these cows to be markedly lower than those concentrations of carotene in cows fed the control ration containing no soybean products. These differences, in both trials, tended to level off after about 6-8 weeks of feeding the beans.

Measurable differences occurred between the blood plasma carotene concentrations of cows fed soybean oil (1.7 lb. daily per cow) and the controls, the cows getting the soybean oil having the lower concentration. These differences were not so large as those caused by raw soybeans. Soybean oil meal caused no great change in carotene concentration in either the blood plasma or milk fat from the control ration. Raw soybeans and soybean oil caused differences in the concentrations of carotene in milk fat which were similar in direction to those found in the blood plasma when these feeds were compared with a control ration. The rations caused small variations, with no particular trends in the vitamin A concentrations of the blood plasma and in the milk fat.

- P15 Further Studies on the Relationship between the Feeding of Soybeans and the Vitamin A Requirements of Dairy Cattle. M. F. ELLMORE, J. C. SHAW, AND B. C. HATZIOLOS, University of Maryland, AND L. A. MOORE AND J. F. SYKES, Bureau of Dairy Industry, U. S. Department of Agriculture.

Calves fed soybeans heated to 100° C. for 15 minutes exhibited a lower plasma vitamin A and a lower liver vitamin A than control calves which received an equivalent amount of raw soybeans. The feeding of 1 g. of iodinated protein per 100 lb. of body weight did not prevent the decrease in plasma vitamin A. Ayrshire and Holstein calves receiving 32 γ of carotene per lb. of body weight exhibited an increased spinal fluid pressure when soybeans constituted 30 per cent of the ration. These animals also exhibited testicular degeneration. Data will be presented on blood plasma and liver vitamin A levels of calves on a soybean ration in which the carotene was replaced by a vitamin A concentrate.

- P16 The Influence of Tocopherols on the Fat Content of Milk. F. WHITING AND J. K. LOOSLI, Cornell University.

Experiments using 16 dairy cows representing the Holstein, Guernsey, and Brown Swiss breeds were carried out to study the influence of tocopherols (vitamin E) and cod-liver oil upon butterfat production. When tocopherols were fed at the rate of 1 g. per cow daily over a 4-week experimental period during winter feeding, the percentage fat in the milk was increased slightly. Cod-liver oil fed at the rate of 5 ounces per cow daily decreased the fat percentage approximately 11 per cent. Feeding tocopherols to cows fed cod-liver oil did not prevent the fall in butterfat percentage. Total milk production (lb. of 4% F.C.M.) was not significantly affected by feeding either tocopherol or cod-liver oil. Feeding the same amount of tocopherol to cows on pasture slightly increased the fat test of the milk but had no influence upon total milk production. Feeding toco-

pherols alone or in combination with cod-liver oil increased the tocopherol content of the butterfat produced, but had no apparent influence upon the vitamin A or carotene content of the fat. Feeding cod-liver oil increased the vitamin A content but decreased the tocopherol and carotene content of the butterfat.

P17 Covitamin Studies of the Milk Fats from Four Breeds of Dairy Cattle. V. N. KRUKOVSKY AND F. WHITING, Cornell University.

A study was made of the relationship between the tocopherol, vitamin A and carotenoid content of milk fat from four breeds of dairy cows. The analyses were made on individual fat samples obtained from 40 cows at the end of pasture feeding and again after 5 months of winter feeding.

Large variations were found in the content of these vitamins between individual cows of the same breed, between the different breeds, and between seasons. The following average values and standard deviations of tocopherols, carotenoids and vitamin A were found for each of the following breeds at the end of pasture season (all values $\gamma/100$ g. of fat): Holsteins, $2,253 \pm 822$, 504 ± 249 , and 546 ± 145 ; Brown Swiss, $2,860 \pm 656$, 785 ± 221 , and 703 ± 146 ; Guernseys, $3,164 \pm 462$, $1,583 \pm 237$, and 381 ± 120 ; and Jerseys, $3,036 \pm 498$, $1,236 \pm 358$, and 578 ± 123 , respectively. After 5 months of winter feeding, the corresponding values were as follows: Holsteins, $2,011 \pm 341$, 290 ± 109 , and 398 ± 86 ; Brown Swiss, $2,149 \pm 487$, 341 ± 165 , and 383 ± 47 ; Guernseys, $2,329 \pm 343$, 772 ± 169 , and 312 ± 109 ; and Jerseys, $1,905 \pm 361$, 341 ± 108 , and 301 ± 26 , respectively.

A highly significant correlation was found between the tocopherol and carotenoid content of the fat. However, no such relationship existed between tocopherol and vitamin A.

P18 The Effect of Lactation and Gestation on Heat Production and Cardiorespiratory Activities of Dairy Cattle and Rats.¹ SAMUEL BRODY, D. M. WORSTELL, A. C. RAGSDALE, AND H. H. KIBLER, University of Missouri.

Heavily lactating cattle and rats produce about twice as much heat under normal feeding conditions as those not lactating. Gestation increases heat production in cattle and rats about 40 per cent above the non-gestating level but only during the last third of the gestation period. The course of pulse rate, respiration rate and ventilation rate parallels the course of heat production. However, the tidal air tends to decline during the last third of gestation.

¹ Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 1111.

- P19 A Biochemical and Histo-pathological Study of Ketosis in Dairy Cattle. J. C. SHAW, B. C. HATZIOLOS, AND V. P. SAARINEN, University of Maryland.

Blood samples were drawn from five cows which appeared to have ketosis and the animals were slaughtered for chemical and histopathological studies of various organs. Blood hemoglobin, red cell volume, phosphorus and chlorine were normal. Blood plasma cholesterol was low. The adrenals were low in ascorbic acid and normal in cholesterol. The total fat of the adrenals was high in all cases. Four of the five livers were high in fat and the kidneys were comparatively high in fat. The increased fat content of these organs was principally neutral fat, the cholesterol and phospholipid fractions being comparatively low. Liver glycogen was low in four of the five cases. The ascorbic acid of the adrenals was low, whereas the cholesterol was normal. Blood counts were made in three cases. The neutrophiles were higher than normal and the lymphocytes and eosinophiles low. The adrenals all exhibited marked fatty infiltration and partial degeneration, the heaviest degeneration being in the outer layer of the cortex, the arcata. The medulla of the adrenal was not affected. Marked fatty infiltration and partial degeneration also were observed in the pancreas, thyroids and the growing follicles of the ovary. One pituitary showed a rupture in the wall of the residual lumen resulting in a small sac filled with colloidal fluid. In another a depression was observed in the inferior part of the anterior lobe, apparently caused by outside pressure and resulting in a partial atrophy of the lobe. Data also will be given on paraffin sections. This constitutes a progress report only.

- P20 A Study of Sampling at Various Stages of Milking in Determining the Bacterial Flora of the Udders of Dairy Cows. E. M. KESLER, C. B. KNOTT, AND J. J. REID, The Pennsylvania State College.

Strict foremilk was compared to samples obtained after various amounts of milk had been removed from the quarters. Nine hundred and forty samples were collected from 47 cows of varying age, production, stage of lactation and types of udder bacterial flora based on previous examinations. After careful washing of the udders with chlorine solution, five 15-ml. samples were drawn aseptically from each quarter as follows: strict foremilk, followed by two successive samples, midmilk, and strippings. Samples were incubated with brilliant green-sodium azide and then examined microscopically by means of a modification of the standard direct count method. All samples were streaked on Edwards medium, incubated, and examined for nature of growth if present. Further tests to identify the various streptococci found on the plates were run as necessary. These included isolation and cultivation in veal infusion broth, followed by

physiological and serological studies. No pronounced differences existed between strict foremilk and the second 15 ml. drawn from the teat. Not all of the long chain streptococci would have been noted had a sample of midmilk or strippings been used as the basis of the tests. Excessive numbers of leucocytes appeared more often in the last-drawn samples.

P21 A Permanent and Convenient Rumen Fistula for Dairy Cows. G. E. STODDARD AND N. N. ALLEN, University of Wisconsin.

Permanent rumen fistulas provide a very satisfactory means of studying digestion in the rumen, but there has been a need for a closure which will prevent leakage but which may be removed readily for sampling or observation. A cannula has been designed for this purpose. It is constructed entirely of transparent leucite, which is easily tooled and fabricated and at the same time resists corrosion and is non-irritating to the contingent tissue. A detachable base flange is threaded to the cannula to facilitate insertion, and a threaded collar is provided for adjusting the outer flange. A screw cap covers the 2-inch opening and is easily removed for observation or sampling. Special tools have been designed for insertion of the cannula at the time of fistulation.

Four cows have been fistulated and equipped with these cannulae. Leakage around the cannula has been slight. Three of the cows were fistulated during early pregnancy. One has freshened normally, and the other two are nearing termination of normal pregnancy. Cannulae of similar design have been used for one goat and a number of sheep.

P21-A Studies Bearing on the Bloat Problem. H. H. COLE, AND MAX KLEIBER, University of California.

In four 4-hour test periods, there was an average consumption of 47.6 lb. of sudan tops fed *ad libitum* in the barn with an average ruminal gas production of 4.7 cubic feet for the period. In four tests with alfalfa tops in the prebloom stage fed *ad libitum*, there was an average consumption of 15.5 lb. and an average of 4.3 cubic feet of ruminal gas produced. The rate of gas formation following the introduction of glucose into the rumen through a cannula has been compared with that of starch. When the cow was fed 9 lb. of alfalfa hay and 6 lb. of barley 20 hours before the experimental period, glucose caused a more rapid increase in gas formation than did starch, although the total gas produced within 4.5 hours after the introduction of either of the two substances was approximately the same, 3.4 and 3.1 cubic feet, respectively.

From pasture consumption studies, evidence was obtained indicating that alfalfa becomes more palatable to cattle as it approaches the bloom stage. Alfalfa in different fields at comparable stages of growth varied widely in palatability.

P22 Calf Losses in a Self-contained Herd Over a Period of Seventeen Years. R. E. JOHNSON, E. L. JUNGHERR, AND W. N. PLASTRIDGE, Storrs Agricultural Experiment Station.

The data for this study were derived from post-mortem examination of 78 calves, representing a mortality of 7.24 per cent, which died prior to the age of 6 months in the University of Connecticut dairy herd during the 17-year period 1931 through 1947. Forty-one of these calves were males and 37 females. Each cadaver was subjected to pathological and bacteriological examination for diagnosis.

White scours, respiratory diseases, bloat and weakness at birth were the principal causes of death aside from miscellaneous conditions which accounted for 19 per cent of the mortality. White scours, responsible for 43.6 per cent of the losses, usually was observed during the first 6 days following birth; all but three of the 34 calves in this group died on or before the ninth day. The average age at time of death from white scours was 6.6 days. In a majority of cases, coliform organisms were isolated from either the cardiac blood, liver, spleen, navel artery or other internal organs. Cases showing gross evidence of navel infection occurred in this group. Respiratory conditions were responsible for 20.5 per cent of the losses. The average age at death in 16 cases was 47 days. Streptococci, staphylococci or pathogenic diphtheroids were isolated. Bloat was responsible for 9 per cent of the deaths, the ages of the calves ranging from 35 to 85 days. Weakness at birth accounted for 7.7 per cent of the deaths, which occurred at an average age of 6.2 days.

Although all calves up to 6 months of age were considered, no calves died after the first 93 days. There was no apparent seasonal effect on mortality when all causes of death were considered, but a slight tendency toward a higher incidence of white scours was observed during the period from August through January.

P23 The Effect of Prepartum Vitamin A Supplementation on the New-born Calf. A. A. SPIELMAN, H. D. EATON, J. K. LOOSLI, AND K. L. TURK, Cornell University.

Work has been done to determine the effect of supplementing the dry-cow ration with one million I.U. of vitamin A daily for 30 days prior to parturition on health and performance of the newborn calf. All cows received a commercial fitting grain mixture (12 per cent protein), mixed grass legume hay and corn silage. One group of 19 cows received only this standard dry-cow ration. Another group of 14 cows received the standard ration plus alfalfa leaf meal as a source of vitamin A. A third group of 9 cows received the standard ration and the alcohol form of vitamin A, while the fourth group of 16 cows received the standard ration

and the ester form of vitamin A. The calves from these cows remained with their dam for the first 2 days after birth. They were fed their mother's milk for the first week and herd milk thereafter. Calf starter, hay and water were fed *ad libitum*.

Calves from the dams fed the alcohol form of vitamin A were significantly higher in plasma carotenoids for the entire experimental period than calves from the other three groups. Calves from dams supplemented with the ester form of vitamin A were significantly higher in plasma carotenoids than those calves from dams fed alfalfa leaf meal. At any one age no significant differences were found between groupings. Calves within a grouping varied significantly among themselves during the experimental period and with age.

Calves from dams fed either form of vitamin A were significantly higher in the level of plasma vitamin A for the entire experimental period than calves from control dams or from dams fed alfalfa meal. There were no real differences between either group of calves from vitamin A-supplemented dams or between calves from control dams and calves from dams fed alfalfa. The significant differences cited above also existed at birth, 3 weeks of age, and 4 weeks of age. Calves within an experimental grouping showed significant differences in the blood plasma vitamin A levels with age and among themselves.

No statistical differences were found in the feed consumed by the various groups. Calves from dams fed either form of vitamin A were significantly heavier throughout the entire experimental period than calves from control dams or from dams fed alfalfa meal. In addition, calves from dams fed alfalfa meal were significantly heavier than calves from control dams. At any one age, the only statistical difference was at 4 weeks, when calves from dams fed the ester form of vitamin A were significantly heavier in liveweight than calves from control dams.

The calves from dams fed both forms of vitamin A had significantly less scours than calves from control dams. No real differences were found between calves from dams fed vitamin A and dams fed alfalfa meal.

P24 The Utilization of Fetal Liver Stores of Vitamin A by the Newborn Calf. A. A. SPIELMAN, H. D. EATON, R. E. JOHNSON, L. D. MATTERSON, AND R. J. SLATE, University of Connecticut.

Studies have been made to determine whether or not the vitamin A stored prenatally is utilized by the newborn calf. Calves from cows fed a standard dry-cow ration and calves from dams fed the same ration supplemented daily for 30 days prepartum with one million I.U. of vitamin A and 5 g. of soybean lecithin were removed from their dams at birth and fed reconstituted skim milk. Plasma carotene and vitamin A were determined

at birth and daily thereafter. The calves were slaughtered on the tenth day and the livers were analyzed for carotene and vitamin A.

At birth, calves from the vitamin A-supplemented cows had higher plasma vitamin A levels, which increased with age, whereas no such increase was noted in calves from the non-supplemented dams. At the end of 10 days the vitamin A remaining in the livers of the calves from vitamin A-supplemented cows was higher than the amount in the livers of the calves from non-supplemented cows. No appreciable differences between the two groups of calves were found in the carotene content of either plasma or liver.

P25 Effect of the Method of Administration of Carotene and of Vitamin A upon the Rate at Which They Are Absorbed from the Alimentary Tract of Dairy Calves. N. L. JACOBSON, G. H. WISE, AND R. S. ALLEN, Iowa State College.

At minimum intervals of about 1 week, carotene and vitamin A concentrates were added to the rations of young dairy calves to determine the effect of method of administration upon the rate of absorption of these substances from the alimentary tract. Milk, into which a vitamin supplement was homogenized, was fed to a series of paired individuals alternately by a nipple pail and by a stomach tube introduced into the rumino-reticular cavity. In a second series of calves, administration of the vitamin supplement by capsule was compared with nipple pail feeding of the homogenized product.

Results indicate that the rates of absorption, as measured by the concentrations of the carotene and vitamin A in the blood plasma, were more rapid when these substances pass directly to the abomasum than when they enter the rumino-reticular cavity. The absorption rate following administration of carotene in capsules indicated passage into the rumino-reticular cavity before entering the abomasum. The physiological significance of these findings has not been established, but it would seem possible that direct passage to the abomasum may enhance efficiency of utilization. The nature of the changes in blood plasma carotene and vitamin A levels following oral administration of carotene suggests a partial conversion of carotene to vitamin A in the intestinal wall.

P26 Some Irregular Fluctuations in the Vitamin A Level of Blood Plasma Produced by Ration Changes in Calves. W. C. JACOBSON AND J. W. THOMAS, Bureau of Dairy Industry, U. S. Department of Agriculture.

Weekly vitamin A determinations were made on 33 calves receiving a ration consisting of a limited quantity of whole milk to 60 days, a grain mixture and alfalfa hay. Six calves received 25,000 I.U. of vitamin A per day for the first 30 days; 12 received 50,000 I. U. of vitamin A per day for

varying periods of time; 15 received no supplemental vitamin A. At 90 days of age, all calves were placed on a vitamin A-deficient ration consisting of grain, skim milk, and wood shavings.

In general, there was a relationship between plasma vitamin A level and the vitamin A intake at these levels of supplementation. With this existing relationship it would seem that a reduction in the vitamin A intake by placing the calves on the deficient ration would cause a similar reduction in the level of plasma vitamin A. Instead, of 12 calves which received 50,000 I.U. per day, seven showed a decided increase in the vitamin A in the blood plasma. Two of the calves in the 25,000 I.U. group showed a slight increase, which persisted for 2 to 7 weeks. The plasma vitamin A level in the non-supplemented group gradually dropped to a low level when the calves were placed on the deficient ration. The reasons for the increase in the plasma vitamin A are not known. However, a relatively large store of vitamin A, along with the introduction of skim milk to the ration, may have been responsible for the increase in the plasma vitamin A levels.

P27 The Influence of the Ration on Some of the Blood Vitamin Changes in the Young Dairy Calf. J. W. HIBBS AND W. D. POUNDEN, Ohio Agricultural Experiment Station.

The plasma carotenoid level in calves fed whole milk and alfalfa hay from birth was observed to be markedly higher during the first 6 weeks than the levels in calves fed whole milk alone, whole milk plus grain, or whole milk plus grain plus alfalfa hay.

The inoculation of the rumens of the calves with rumen microorganisms from cows did not affect markedly the plasma carotenoids or vitamin A changes. The calves which had the highest plasma carotenoid levels maintained the lowest plasma vitamin A levels, indicating an inverse relationship between plasma carotene and vitamin A under these conditions. Liver storage probably complicates the plasma vitamin A level as a measure of vitamin A metabolism.

No marked variations were noted in the plasma ascorbic acid levels between groups fed whole milk and alfalfa hay and whole milk plus grain plus alfalfa hay. However, a higher, more uniform level of plasma ascorbic acid was maintained during the first 6 weeks in the calves which were inoculated with rumen microorganisms than in those not so inoculated.

These results, when correlated with those in paper P28, emphasize the value of good quality hay in meeting the vitamin needs of young calves through the establishment of early rumen function.

P28 The Influence of the Ration on the Digestive Tract Microorganisms of the Young Dairy Calf. W. D. POUNDEN AND J. W. HIBBS, Ohio Agricultural Experiment Station.

The development in the rumens of young calves of protozoa and bac-

teria of the types associated with alfalfa hay ingestion was helped by inoculation with microorganisms from rumens of mature stock, provided the calves were ingesting a sufficiently high proportion of hay. The numbers of protozoa increased as the proportion of grain increased in relation to the quantity of alfalfa hay ingested, until approximately equal parts of each were being consumed. Further increases in the proportion of grain were accompanied by reduction in numbers of both protozoa and hay-type flora until they eventually disappeared.

Bacteria of the types associated with grain ingestion made their appearance in appreciable numbers in samples once the proportion of grain consumed exceeded the hay; they continued to increase as the proportion of grain increased. Their development was not influenced by the inoculations. Indications are that the early development of rumen microorganisms similar to those observed in cows is influenced by the feeds consumed. In addition to the beneficial effect on certain blood vitamin constituents (see paper P27), the general appearance of the calves fed milk and hay alone was improved by rumen inoculation.

P29 Relation of Aerobic Bacterial Flora to the Consistency of the Feces.

M. D. VAN PELT, R. E. JOHNSON, AND W. N. PLASTRIDGE, Storrs Agricultural Experiment Station.

The development of the bacterial fecal flora was followed in 26 calves through the first 19 days of life with special attention to *Escherichia coli*. The physical characteristics of the daily fecal samples were recorded with the rectal temperature of the calf. The total bacterial count and the gram-negative count were determined with differential media.

Calves are born with a sterile alimentary tract which rapidly becomes contaminated. The increase in bacteria is very great during the first 24 hours and, in calves raised according to a normal herd procedure, the flora tends to reach a peak on the second day. It then decreases slowly for 14 days, when it levels off. There is a seasonal difference in calves, with the fall calves having a significantly higher flora than summer calves.

The consistency of the feces goes through three phases: (a) the meconium, which is brown and elastic, lasting for 1 day; (b) the transitional stool, which is slimy in consistency and varies from light yellow to green in color, lasting about a week; and (c) the normal phase, which is dark brown, soft yet firm.

There was no significant correlation between high temperature or diarrhea and an abnormally high flora or a high ratio of *E. coli* to the total bacterial count.

Six calves were placed on skim milk at birth. All had periods of scouring and produced a transitional stool throughout the experimental period. Two calves died of white scours and *E. coli* was isolated from their liver,

kidney and spleen. During the actual periods of scouring, the flora was much lower than for previous samples. However, the flora was abnormally high 1.5 to 2 days before the scouring began. The flora of the calves fed skim milk was significantly higher throughout the period of observation than that of the normal calves.

P30 Raising Dairy Calves Without Colostrum. J. T. MILES, S. A. HINTON, AND HOMER PATRICK, Tennessee Agricultural Experiment Station.

Since prepartum milking is gaining in popularity as a means of relieving congestion in the cow's udder at calving time, and since valuable brood cows, because of disease or injury, may fail to produce utilizable colostrum at calving time, there is need for a method of raising calves without colostrum. Dairy bull calves born at the Knoxville Station were divided into three groups and fed as follows: Group I, dam's colostrum and milk for first week; Group II, a laxative at birth and herd milk fortified with vitamin A from birth to 1 week of age; Group III, a laxative at birth and herd milk fortified with a mixture of pure vitamins from birth to 1 week of age. All groups received the same kind of milk and other feeds after they reached the age of 7 days.

The six calves in Group I and eight calves in Group III have lived and made normal gains. Of the nine calves in Group II, four died before they reached the age of 30 days; others in this Group made subnormal gains.

P31 A Comparison of Corn Starch, Dextrin and Corn Sugar as the Principal Carbohydrate Source in Synthetic Rations for Calves. R. J. FLIPSE, C. F. HUFFMAN, C. W. DUNCAN, AND F. THORP, JR., Michigan State College.

Thirteen calves were divided into three groups and placed on synthetic milk diets at ages of 3 to 5 days. Group I received corn starch (dextrose), Group II dextrin, and Group III corn sugar as the principal carbohydrate source in the ration. Rations were identical except for the carbohydrate component. Twelve vitamins were added to the ration.

Weekly blood analysis showed normal red cell volume, hemoglobin, and magnesium but low levels of plasma calcium, inorganic phosphorus and ascorbic acid as compared to the values for the blood of similar calves on normal rations.

Growth was subnormal in all calves, indicating inadequacy of the ration. After 4 weeks on synthetic rations, the average change from the starting weight was +3.3 per cent, -8.8 per cent and -6.9 per cent for the sugar, dextrin, and starch groups, respectively. Calves on sugar showed a sleeker coat of hair and decidedly less tendency to scour than did calves of the

other two groups, but paralysis, curable by either potassium or biotin, affected all calves on sugar and none on dextrin or starch. The average survival time was 31.0, 16.6 and 31.3 days for the sugar, dextrin and starch groups, respectively; however, the average starting age was 13.7 days on starch as compared to 4.6 on dextrin and 3.8 on sugar.

Necropsy characteristically revealed no abnormalities outside the gastro-intestinal tract. Petechial hemorrhages in the abomasal mucosa were the most common finding; these were most numerous in the dextrin group but also appeared in the starch and sugar groups. Congestion of the duodenum and of the colon was prevalent in calves fed starch and dextrin but seldom was observed in calves on sugar.

P32 Effect of Tryptophan in the Diet on the Excretion of Niacin and Its Metabolic Products by Dairy Calves. G. C. ESH AND T. S. SUTTON, The Ohio State University and Ohio Agricultural Experiment Station.

Very young calves, fed a nicotinic acid-free ration, showed no deficiency symptoms (J. Biol. Chem., **167**: 729-736). Since the proteins of colostrum and milk are generously supplied with tryptophan, the possibility that dietary tryptophan may affect the urinary excretion of nicotinic acid and its metabolic products was investigated.

Five grams of L-tryptophan were fed daily for 3 days to each of two calves that had been maintained from birth on a whole milk diet. The urinary excretion of nicotinic acid, its metabolic products and tryptophan were determined on 24-hour samples and compared with similar data obtained previous to and following the tryptophan feeding.

The data indicate that N¹-methyl-nicotinamide is not the main metabolic product excreted by calves. Tryptophan feeding resulted in a 3-fold increase in the excretion of total free and combined nicotinic acid. There was little change in the excretion of free nicotinic acid and N¹-methyl-nicotinamide. The major portion of the increase was in the non-methylated products. The urinary excretion of tryptophan accounted for only 1 to 1.5 per cent of the intake. The data show that increases in dietary tryptophan result in increased urinary excretion of total nicotinic acid, indicating that tryptophan serves as a precursor of niacin in the calf as in other mammals thus far studied.

P33 Performance of Calves on a Photolyzed Milk Diet. R. G. WARNER AND T. S. SUTTON, The Ohio State University.

A recent report of riboflavin deficiency in calves fed a riboflavin-free synthetic milk (J. Nutrition, **33**: 263. 1947) prompted a study of the response of calves to a diet of milk in which the major part of the riboflavin

had been photolyzed. Approximately 96 per cent of the riboflavin in the milk fed was destroyed by exposure to the radiations of a 400 W mercury vapor lamp emitting light of wave lengths longer than 3000 Å. Four male Guernsey calves were fed the treated milk supplemented with vitamin A. One of these calves also received approximately 2.99 mg. of added riboflavin daily.

Deficiency symptoms consisted of intermittent diarrhea, a scurfy skin condition, alopecia, periodic excessive salivation and lacrimation, and, in the acute stages, difficulty in swallowing. These calves were extremely unthrifty. The addition of 2 mg. riboflavin daily to one of these calves resulted in a prompt cessation of diarrhea, resumption of growth, and marked improvement in general appearance, including the growth of new hair.

The performance of the calf receiving 2.99 mg. of added riboflavin from the start was uneventful and approached the Ragsdale standard in growth. Blood vitamin A and ascorbic acid levels were normal in all four calves. The urinary excretion of riboflavin varied between 0.01–0.06 mg. per day for calves receiving no added riboflavin and 0.38–0.64 mg. per day for the calf which received added riboflavin throughout the experiment. Data indicate that riboflavin requirement of a 70-lb. calf, is somewhat less than 3 mg. daily, when fed an exclusively whole milk diet.

P34 Anemia in Young Calves and Its Alleviation by Iron. W. C. JACOBSON AND L. A. MOORE, Bureau of Dairy Industry, U. S. Department of Agriculture.

Anemia has been observed to occur in calves in the various herds at Beltsville. The anemia may be present at birth or may develop at any time up to 60 days of age. However, it usually corrects itself at about 80 or 90 days of age. About an equal percentage of calves from each of the breeds had hemoglobin values below 8 g. per 100 ml. except the Sindhi-Jersey crosses, which had a very high hemoglobin level for the first 90 days.

When a salt mixture of cupric sulfate, ferric sulfate, manganese chloride and cobalt sulfate was fed, the anemia was alleviated. When each of these minerals was fed separately, it was found that iron was the only one which alleviated the anemia. Red cell counts and red cell volumes were run along with the hemoglobin determinations on some of the calves receiving iron. After the calf had received iron for 2 weeks, there was an increase in the red cell count and an increase in the volume of the red cells as well as an increase in the quantity of hemoglobin in the blood. The weight gains to 90 days of age were studied. The calves with the lower hemoglobin values gained less on the average, but this difference was not statistically significant with the number of calves involved. However, the group of calves which had low hemoglobin values showed more variation in their weight gains.

P35 A Method of Evaluating Bull Semen. T. M. LUDWICK, D. OLDS,
AND MARSHALL CARPENTER, Kentucky Agricultural Experiment
Station and Kentucky Artificial Breeding Association.

Samples of diluted semen were incubated at 100° F. and observed under the microscope at regular intervals until motility ceased. Observations were made on data summarized from the Kentucky Artificial Breeding Association and cover a period of 11 months. The observations include 305 ejaculates from 27 different bulls of the Holstein, Jersey and Guernsey breeds from which approximately 12,000 cows were bred.

The coefficient of correlation between incubation time (time for sperm to lose all activity when held at 100° F.) and conception rate (based on 60-90 day non-returns) was 0.84 ± 0.03 when only ejaculates which were used in the breeding of as many as 30 or more cows were included. Ejaculates which were used to breed only a few cows did not give good correlations.

P36 Vital Staining of Bovine Spermatozoa with an Eosin-aniline Blue Staining Mixture. H. E. SHAFFER AND J. O. ALMQUIST, The
Pennsylvania State College.

In 1942 Lasley, *et al.* (Anat. Record, 82: 167-174) reported upon an opal blue-eosin staining mixture for the differentiation of live and dead ram spermatozoa. Since opal blue was unobtainable, studies were undertaken in this laboratory to determine whether readily available dyes could be used. Aniline blue provided a suitable substitute for opal blue as the background stain. When used in combination with either eosin yellowish or eosin bluish, a satisfactory mixture for differential staining of bovine spermatozoa was obtained.

To determine the optimum concentrations of the dyes, seven levels of both eosin Y and eosin B (0.8 to 2.5 per cent) were tested in combination with 2, 3, 4 and 5 per cent aniline blue. The dyes were dissolved in a citric acid-disodium phosphate buffer, and five ejaculates of freshly collected bull semen were used to compare the staining properties of the 56 solutions. Four slides were prepared from each ejaculate using each of the staining solutions, and 100 sperm cells were counted per slide. Based on the quality and uniformity of the slides and the percentages of unstained spermatozoa (presumed to be living), 1 per cent eosin and 4 per cent aniline blue were selected.

Studies were conducted to determine the effects of concentration of buffer salts and pH of the buffer used to dissolve the dyes. Five concentrations of phosphate buffer (M/4 to M/12) were adjusted to pH 5.6, 6.4, 7.2 and 8.0, and 1.0 per cent eosin and 4.0 per cent aniline blue were added to each of the solutions. Differences in the percentages of unstained cells due to buffer concentrations were not statistically significant, while those due to

pH were significant at the 5 per cent level. When the appearance of the slides also was considered, a final staining mixture consisting of 1 per cent eosin *B* and 4 per cent aniline blue dissolved in M/8 phosphate buffer having a pH of 7.2 was selected as a satisfactory differential stain for bull spermatozoa.

A simple technique for preparing uniform slides has been developed. The slides are dried on a hotplate maintained at a temperature of 85 to 100° C. and an electric fan is used to hasten the drying process. Field trials now are in progress to determine the value of this staining method as a measure of semen quality.

P37 Turbidometric Assay of Hyaluronidase in Bull Semen. JOHN P. MIXNER AND JAMES E. JOHNSTON, New Jersey Agricultural Experiment Station.

Hyaluronidase (*h*-ase) is an enzyme found in bull semen which has the ability to depolymerize hyaluronic acid. Hyaluronic acid is an important constituent of tissue cell cement. The turbidometric assay for *h*-ase is based on the discovery that the turbidity produced by the interaction of hyaluronic acid and acidified blood serum is, within limits, a function of the hyaluronic acid concentration. The conventional unit of *h*-ase, the turbidity reducing unit (TRU), is not comparable between laboratories due to variations in serum protein concentrations, hyaluronic acid preparations and pH control in assaying. Comparative assaying of *h*-ase is greatly facilitated by assaying in terms of a standard preparation of *h*-ase which may be exchanged among laboratories. The coefficient of correlation secured between milligrams of standard *h*-ase and colorimeter reading was -0.985 ± 0.006 . The standard error of estimate of the regression equation gives an error of ± 8 per cent on the mean value of milligrams of *h*-ase.

H-ase assay values of semen in terms of milligrams of standard *h*-ase, secured by diluting semen at various rates, conformed to the standard regression line of purified *h*-ase. This is important since the concentration of salts, particularly NaCl, influences greatly the activity of *h*-ase. The data for 117 semen assays were calculated both in TRU's and in milligrams of standard *h*-ase. A coefficient of correlation of $+0.954 \pm 0.028$ was secured between the two, indicating the essential sameness of the two measures of *h*-ase potency.

P38 Hyaluronidase and Bull Semen. J. E. JOHNSTON, E. J. STONE, AND J. P. MIXNER, New Jersey Agricultural Experiment Station.

The enzyme hyaluronidase (*h*-ase) is capable of denuding the mammalian ovum of follicular cells adhering after ovulation. Therefore, a relationship between *h*-ase and fertility has been postulated.

An investigation of bull semen in relation to *h*-ase has shown: (a) that *h*-ase is present immediately after ejaculation, (b) that the concentration of *h*-ase in the seminal plasma increases on incubation of the semen at either 5 or 37° C., (c) that a maximum potential concentration of *h*-ase is obtained on incubation of semen for 24 hours at 37° C. under toluene, and (d) that *h*-ase seems to be tied up with the spermatozoa, since incubation of sperm-free seminal plasma yields no increase in *h*-ase concentration. On this basis, procedures for determining semen *h*-ase potency were adopted in which semen samples were assayed initially, within an hour after ejaculation, and again after 24 hours incubation at 37° C. under toluene.

One hundred semen samples were analyzed; a coefficient of correlation of $+0.639 \pm 0.060$ was obtained between initial and 24-hour *h*-ase concentrations. The following coefficients of correlation were obtained between initial *h*-ase concentrations and other semen characteristics: sperm concentration per mm³, $+0.540 \pm 0.072$; initial motility, $+0.0425 \pm 0.101$; volume of ejaculate, $+0.157 \pm 0.095$; sperm per ejaculate, $+0.479 \pm 0.078$; and duration of second motility, $+0.0132 \pm 0.101$. Twenty-four-hour *h*-ase concentrations gave the following coefficients of correlation: sperm concentration per mm³, $+0.697 \pm 0.052$; initial motility, $+0.245 \pm 0.095$; volume of ejaculate, $+0.206 \pm 0.097$; sperm per ejaculate, $+0.540 \pm 0.072$; and duration of second motility, $+0.0018 \pm 0.101$.

P39 The Effect of Testis Biopsy on Semen Characteristics. J. F. SYKES, W. J. SWEETMAN, P. C. UNDERWOOD, AND L. A. MOORE, Bureau of Dairy Industry, U. S. Department of Agriculture.

A marked reduction in sperm concentration resulted when testis biopsies were performed on three bulls. Two biopsies were performed on each animal, and a progressive drop in sperm concentration occurred with succeeding biopsies in every instance. The first biopsy had little or no effect on other sperm characteristics, but after a second biopsy both initial and storage motility were reduced.

Microscopic examination indicated that widespread damage occurs in the germinal epithelium following biopsy. Repair appeared to occur, but in spite of this apparent recovery there was no increase in sperm concentration even after intervals of 6 to 15 months.

P40 Spermatozoa Behavior in Bovine Cervical Mucus at Varying Stages of Estrus.¹ H. A. HERMAN AND OTIS H. HORTON, University of Missouri.

To gain additional information on factors affecting the conception rate in dairy cattle, a study of the mucus secretions of the cervix and uterus has

¹ Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 1109.

been undertaken. The viscosity and penetrability of mucus are factors in spermatozoa movement through the uterus and up the tubes. The consistency of mucus changes during the estrus period. In early heat it is thin and watery; as heat progresses it becomes more viscous and there is an infiltration of leucocytes.

The properties of cervical and vaginal mucus were studied with respect to flow-elasticity, penetrability of spermatozoa, volume, pH and leucocyte number. Vaginal temperatures also were recorded. The viscosity of cervical and vaginal mucus was found to be lowest during the first 6 hours of estrus and gradually increased as estrus was prolonged. Penetrability of the mucus by spermatozoa was highest during the first 6 to 10 hours of heat. The volume of mucus was greatest during the first part of the heat cycle. There was a rise in vaginal temperatures during estrus, the peak temperature generally occurring 14 to 16 hours after the beginning of the heat period. The mean pH of mucus *in vivo* was found to be 7.23 during estrus and ranged from 7.25 to 7.39 during non-estrus.

The results in these preliminary studies indicate that the properties of bovine mucus may be of considerable interest in determining the most satisfactory practices to follow in obtaining a high conception rate where artificial insemination is used.

P41 Varying the Proportion of Egg Yolk in Diluters for Bull Semen.

ERIC W. SWANSON, University of Tennessee.

A study was made of the sodium citrate and egg yolk semen diluter to establish optimum concentrations of the respective constituents and to detect the minimum amount of egg yolk which will produce satisfactory results. Concentrations of 1, 2, 3, 4 and 5 per cent of sodium citrate were compared in the egg yolk diluter to determine the least harmful citrate concentration. Concentrations of 2 and 3 per cent were satisfactory, with the latter being slightly superior. This solution also was most nearly isotonic with bull semen. Diluters were prepared with egg yolk and 3 per cent sodium citrate mixed in proportions of 1 to 1, 1 to 3, and 1 to 7. Daily estimations of percentage progressively motile sperm were made on semen diluted identically with the three diluters. Very little difference was noted between the 1 to 1 and 1 to 3 diluters, and the 1 to 7 diluter was very satisfactory although slightly inferior. The ability of freshly collected sperm to withstand temperature shock in the three diluters was checked by means of differential stain for dead and alive sperm before and after shock. The differences between the diluters were small and of doubtful significance. Therefore, a diluter containing one-eighth egg yolk may be used with the advantage of economy of egg yolk and of time and easier microscopic examination.

- P42 A Study of the Types of Bacteria in Bovine Semen and Their Effect Upon Motility.¹ J. E. EDMONDSON, K. L. TALLMAN, AND H. A. HERMAN, University of Missouri.

Since most semen, either diluted or non-diluted, contains variable concentrations of bacteria, the authors have been interested in securing more detailed information regarding the effect of bacteria on the length of storage of bovine semen. The early work consisted of isolating the different types of organisms found in semen. The types isolated included streptococci, staphylococci, micrococci, pseudomonas, bacilli, actinomyces, yeast and *Escherichia coli*. Standard plate counts were made of both diluted and non-diluted semen to determine if there was any correlation between the number of organisms present and the length of time semen could be stored. Careful analysis of the data showed no correlation; however, when samples were plated on blood agar, a definite correlation existed between the number of hemolytic organisms and the length of storage of semen. Using motility as the index of storage, the hemolytic count was found to increase as the length of storage decreased.

The effect of hemolytic and non-hemolytic bacteria upon the length of storage of semen was further proved by studies using fresh diluted and non-diluted semen to which was added a pure culture of organisms previously isolated from semen. Semen samples inoculated with hemolytic bacteria showed no motility after 2 days of storage, while samples containing non-hemolytic bacteria varied in length of storage. Certain non-hemolytic organisms were able to increase the storage time from 1 to 4 days over the controls, while others did not store as long as the controls. It is believed that certain organisms are able to increase storage time of semen either by enzymatic action or by entering into the metabolic processes of the sperm.

- P43 The Effect of Penicillin upon the Fertility of Semen from Relatively Infertile Bulls. JOHN O. ALMQUIST, The Pennsylvania State College.

Previous studies at this Station showed that penicillin inhibited bacterial growth in semen but did not significantly affect the fertility of semen from bulls of relatively high breeding efficiency. To test the effect of penicillin upon the fertility of semen from relatively infertile bulls used in routine artificial breeding, a 5 x 5 Latin square experiment was designed and replicated four times in succession. Penicillin was added to the semen of four Guernsey bulls and one Holstein bull of lowered fertility at the rate of 250, 500, 750 and 1,000 units per ml. of diluter semen with appropriate controls. Data based on 6-month non-returns for 3,576 first and second services demonstrated that the 500- and 1,000-unit levels of penicillin brought

¹ Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 1106.

about highly significant increases in fertility when compared to the untreated controls. Greatest improvement in fertility was obtained with the 1,000-unit level, but the degree of enhancement varied considerably between bulls. Three of the five bulls showed average increases above the controls of 15, 21, and 31 per cent on the basis of 6-month non-returns when penicillin was added at the rate of 1,000 units per ml. of diluted semen. The remaining two bulls showed small increases of 1 and 4 per cent. Apparently only three of the bulls produced semen in which bacteriological control by penicillin was beneficial. The 13-year-old bull which showed the 1 per cent increase was slaughtered because of low fertility 7 months after the close of the experiment. Histological examination revealed marked degeneration of the testes. The untreated semen of the bull which showed a 4 per cent response increased in fertility to such a degree during the experiment that the animal could not be considered a problem bull.

P44 Breeding Results With Bovine Semen Treated With Varying Amounts of Thyroxine. A. B. SCHULTZE AND H. P. DAVIS, University of Nebraska.

Semen samples from 22 bulls, selected at random, were treated with crystalline *d,l*-thyroxine. The relative fertility of this semen was determined from breeding results in artificial breeding associations from the percentage of non-return services after a 4-month period. The conception rate obtained with other semen samples from the same bulls during the same period served as a basis of comparison. Thyroxine was added in the amounts of 2 γ , 10 γ , and 15 γ per 100 ml. diluted semen. The 2- γ level resulted in an increase of 2.2 percentage units in conception rate (732 services, 23 semen samples). The 10- γ level in an increase of 6.4 percentage units (1,847 services, 112 semen samples); and the 15- γ level in an increase of 4.4 percentage units (488 services, 18 semen samples).

P45 Measuring Breeding Efficiency by Pregnancy Examinations and by Non-returns. G. R. BARRETT, L. E. CASIDA, AND C. A. LLOYD, University of Wisconsin.

This report includes data on inseminations made during 1946 in the Wisconsin Experimental Breeding Project, in the area in which routine pregnancy examinations were performed at 35-49 days after breeding. Pregnancy examination information was available for 7,530 inseminations. Of the 4,286 inseminations reported as first services, 52.9 per cent resulted in pregnancies, as determined by pregnancy diagnosis. The 30-60 day non-return percentage for these same inseminations was 67.8, the 60-90 day percentage was 58.4, and the 90-120 day figure was 55.7 per cent. Thus, the discrepancies in favor of the non-return percentages were 14.9, 5.5 and 2.8 per cent. Data concerning services other than the first also will be pre-

sented, as well as a study of monthly variation in the conception and non-return rates.

P46 Order Number of Insemination and Conception Rate. G. R. BARRETT, C. A. LLOYD, AND R. A. CARPENTER, University of Wisconsin.

Inseminations performed in the Wisconsin Experimental Breeding Project from January 24 to May 31, 1945, and from August 13, 1945, to Sept. 30, 1947, were summarized. Pregnancy examinations were performed, usually 35 to 49 days after insemination. An insemination was considered fertile only when an amniotic vesicle could be palpated; inseminations were considered infertile if the cow was rebred or if she was diagnosed not pregnant. Inseminations were excluded if the cow had died, been sold, or could not be examined for any other reason. Of 14,771 inseminations, the number in each group from first to seventh service was 8,621, 3,463, 1,443, 631, 308, 152, and 75, respectively. There were 78 inseminations with order numbers higher than seven. The corresponding percentages of inseminations classed as fertile were 54.4, 50.7, 46.5, 38.2, 36.7, 30.1, 27.7 and 16.7. The mean percentage of fertility for the entire 14,771 inseminations was 51.1.

P47 The Effect of Udder Inflation of Cows with Parturient Paresis, on Blood Calcium, Magnesium and Inorganic Phosphorus. VEARL R. SMITH AND R. P. NIEDERMEIER, University of Wisconsin.

The udders of five Jersey cows¹ in advanced stages of parturient paresis (down and unable to rise) were inflated with air to a pressure of 60 to 70 mm. mercury. A pre-inflation venous blood sample was taken, and post-inflation samples were taken at 0.5, 1.5, 3, 5, 8, 11, 14, 17, 20, 36 and 48 hours. Samples were analyzed for total serum calcium, serum magnesium and plasma phosphorus. Pre-inflation blood calcium, inorganic phosphorus and magnesium ranged from 5.2 to 4.4, 1.4 to 0.5, and 4.5 to 2.0 mg. per cent, respectively. The cows arose of their own volition from 3 to 14 hours after inflation; with one exception, the blood serum calcium was 6 mg. per cent or above at the time the cow arose. The normal phosphorus level had not been reached in four of the five cows by the twentieth hour post-inflation. Three of the five cows had high magnesium levels through the twentieth hour post-inflation.

P48 A Study of Citric Acid Levels in the Blood and Urine of Cows at Time of Parturition. T. H. BLOSSER AND VEARL R. SMITH, University of Wisconsin.

The importance of citric acid in calcium metabolism has been shown by

¹Four of these cows are in the herd of Biltmore Farms, Biltmore, North Carolina. The authors are grateful to the management of Biltmore Farms for making these animals available for the study herein reported.

several workers. Studies have been undertaken to study the relationship of citric acid to the changes that occur in the blood calcium levels at time of parturition. Studies on the blood citric acid and calcium of 18 cows prior, during and subsequent to parturition in general show that as the blood calcium decreases, there also is a decrease in the citric acid. Those cows having parturient paresis at parturition showed much lower levels of blood citric acid than normal cows. Preliminary studies show that there is an increased excretion of citric acid prior to parturition in cows having parturient paresis.

P49 The Effect of Prepartum Milking on Some Blood Constituents of the Cow. R. E. JOHNSON, H. D. EATON, A. A. SPIELMAN, L. D. MATTERSON, AND R. J. SLATE, University of Connecticut.

Two groups of cows, one milked twice daily 10 days prior to the calculated parturition date and the other milked shortly after calving, were used in this experiment. Total hemoglobin, plasma carotene and vitamin A, serum calcium and phosphorus were determined on venous blood samples drawn at weekly intervals beginning 4 weeks before calving, immediately following calving and ending 4 weeks after parturition. In addition, mammary and umbilical edema, expulsion of the placenta, and incidence of clinical milk fever and ketosis were recorded.

No apparent differences in blood constituents were noted between cows milked prepartum and postpartum. Total hemoglobin increased slightly at parturition and thereafter decreased to a slightly lower level than that obtained during the 4-week prepartum period. Both plasma carotene and vitamin A showed a marked drop at the time of parturition and increased thereafter, but they did not attain the same level as that found prepartum. Serum calcium and serum phosphorous dropped appreciably at parturition and returned to the prepartum level within a week after parturition.

To date, due to the limited observations, no significant conclusions can be drawn with regard to the effect of prepartum milking on mammary and umbilical edema, expulsion of the placenta and incidence of ketosis and milk fever.

P50 A Study of Some Blood Constituents of Cows not Milked Following Parturition. R. P. NIEDERMEIER AND VEARL R. SMITH, University of Wisconsin.

Four Jersey cows, not first-calf heifers, were used for this study. Venous blood samples were taken for 5 days previous to the anticipated day of parturition and for 8 days following calving, or until pressure in the udder had subsided. Calves were removed from their dams before being permitted to suckle, and no milk was removed from the udder. Twice-a-

day milking began the seventh day after parturition in one cow, but she never attained a full flow as judged by previous lactations. The other three cows never were milked and later were mastectomized. Blood samples were analyzed for total serum calcium. All four cows showed an appreciable drop in blood calcium on the day of parturition. One cow had parturient paresis 36 hours postpartum. The blood calcium level returned to normal levels by the second day postpartum. Higher-than-normal levels of blood calcium occurred on the third or fourth day postpartum, which seemed to be the time of greatest intramammary pressure.

P51 The Effect of Preparturient Milking on the Composition of Colostrum. A. H. VAN LANDINGHAM, C. E. WEAKLEY, JR., R. A. ACKERMAN, AND GEORGE HYATT, JR., West Virginia Agricultural Experiment Station.

A group of 11 cows and heifers were milked from 3 to 18 days before calving. Samples of preparturient colostrum were taken for chemical analysis when as much as 2 lb. per day was obtained. Total nitrogen, non-casein nitrogen, casein nitrogen by difference, and fat were determined. Samples also were obtained from a comparable group of 11 cows and heifers which had not been milked prepartum.

Colostrum obtained from heifers not previously milked on the day of calving contained an average of 2.34 g. per 100 ml. of total nitrogen, of which 57.3 per cent was in the non-casein nitrogen fraction. By the end of the fourth day following parturition, the total nitrogen was reduced to 0.70 g. per 100 ml., of which only 20.8 per cent was in the non-casein fraction.

The total production of preparturient colostrum varied within the 11 animals from 2 to 142 lb. The total nitrogen and the proportion of non-casein nitrogen to total nitrogen on the day of calving were related to the amount of preparturient colostrum produced. The two heifers producing the most preparturient colostrum secreted colostrum very similar in composition to that of normal milk 1 to 3 days before calving. Other heifers which produced only small amounts of preparturient colostrum before calving produced colostrum after calving very similar in composition to that of heifers which had not been pre-parturient milked.

P52 The Effect of Prepartum Milking on the Carotene and Vitamin A and Proximate Composition of Colostrum. H. D. EATON, A. A. SPIELMAN, R. E. JOHNSON, L. D. MATTERSON, AND R. J. SLATE, University of Connecticut.

A comparison of the carotene, vitamin A, and proximate composition of colostrum of two groups of cows has been made. One group was milked

twice daily for 10 days prior to the calculated parturition date; the other group was not milked until after calving. Calves born to these cows were not allowed to nurse. Pooled aliquot portions of the morning and evening milking for each day prepartum and samples from the first six milkings postpartum were analyzed for carotene, vitamin A, protein, lactose, fat and ash.

The first milking postpartum from prepartum-milked cows was similar in composition to normal milk, especially in those cows milked for at least 10 days before calving. The first milking after calving from postpartum-milked cows contained approximately five times as much carotene and vitamin A, three to four times as much protein, one-half as much lactose, slightly greater amounts of fat, and one and one-fourth times as much ash as the milk obtained from the prepartum-milked cows. These values decreased with successive milkings. Prepartum milking materially alters the composition of the first milk secreted at the termination of pregnancy and produces a milk much lower in nutritive value, as indicated by the analyses.

P53. The Carotene and Vitamin A and Proximate Composition of Portions of the First Milking Postpartum. H. D. EATON, A. A. SPIELMAN, L. D. MATTERSON, R. E. JOHNSON, AND R. J. SLATE, University of Connecticut.

The usual practice is to allow the calf to nurse immediately after parturition. In many cases the cow then is partially milked out. The problem was to establish the relative nutritive value of the successive portions of this first colostrum.

Immediately after calving, cows were completely milked out by 2-lb. increments. The carotene and vitamin A, protein, lactose, fat and ash contents were determined on these samples. Carotene and vitamin A and fat increased with successive increments, while lactose and ash decreased and protein remained essentially the same. Cows milked prepartum and cows milked postpartum gave essentially the same trends.

P54 Effect of the Form of Vitamin A and of Tocopherol Supplements of the Ration on the Concentration of Vitamin A and Carotenoids of Colostrum and Early Milk. D. B. PARRISH, G. H. WISE, AND J. S. HUGHES, Kansas Agricultural Experiment Station.

Determinations were made of vitamin A and carotenoid concentrations of colostrum and early milk from groups of cows that received either a basal ration or a basal ration plus vitamin supplements during the terminal month of gestation. The supplements used were: (a) 0.5-1 million units vitamin A ester, (b) 0.5-1 million units vitamin A alcohol, (c) 0.5-1 million units of vitamin A alcohol plus 0.5-1 g. tocopherols, (d) from 0.5-1 g. to 10 g. of tocopherols.

Wide individual differences were noted in vitamin A and carotenoid contents of colostrum and early milk from cows receiving the various supplements. Consistent differences in the levels of vitamin A did not result from supplementation of the ration with the ester and alcoholic forms of vitamin A. No increase was noted in the vitamin A of colostrum and early milk from cows that received tocopherols in addition to the alcoholic form of vitamin A. Data suggested that tocopherol supplements at high levels might have increased the vitamin A content of colostrum, but due to the small number of animals used, the latter effect was not definitely established. The various supplements had no apparent effect on carotenoid contents of colostrum and early milk.

P55 Comparison of Barn-cured and Field-cured Alfalfa Hay. GILBERT H. ROLLINS AND PAUL M. REAVES, Virginia Polytechnic Institute.

Alfalfa hay was divided into two lots in the field by using alternate windrows. One lot was placed on a barn hay drier when partially cured and curing completed by forced atmospheric air. The other lot was allowed to cure in the field. A double reversal feeding trial was conducted to compare the milk-producing ability of the two hays. Each period was 28 days in length, with a 3-day changeover period. Hay was fed as the sole roughage and grain was fed according to milk production. Twelve cows were used in the trial.

The production of 4 per cent fat-corrected milk was 15,573 lb. while the cows were on the barn-cured hay and 14,994 lb. while on the field-cured hay, or approximately 4 per cent more milk for the barn-cured hay. The decrease in production was more pronounced from the field-cured hay as the trial progressed.

The carotene content of the hay after storage was approximately 60 per cent higher for the barn-cured hay. It was rated as having 62 per cent color and 45 per cent leafiness compared to 47 per cent color and 35 per cent leafiness for the field-cured hay.

P56 Studies on Mow Curing of Baled Hay. W. A. KING, J. W. WILBUR, S. M. HAUGE, AND A. W. COOPER, Purdue University.

Field-cured and mow-cured baled alfalfa hay have been compared in feeding trials for a 2-year period. Unheated air was used to finish the drying of the mow cured hay, which ranged from 30 to 35 per cent moisture when placed in the mow. All the hay was grown in the same field and alternating windrows were used for the field and mow curing. The first-cutting hays contained considerable timothy. Each of the three cuttings of each year were fed to dairy cows during a test period of 9 weeks. The carotene contents of the hays as fed for each of the 2 years were as

follows: first cutting, field—10 and 3 parts per million, mow—13 and 7 p.p.m.; second cutting, field—9 and 2 p.p.m., mow—25 and 15 p.p.m.; third cutting, field—25 and 12 p.p.m., mow—25 and 39 p.p.m. Feeding trials using six cows in each group showed no consistent results in favor of either method of curing. The hay was fed at the rate of 2.5 lb. per 100 lb. of liveweight. It was found that 25 p.p.m. of carotene in the hay would maintain the carotene content of the blood plasma and milk at only a fair level. Hays of 39 p.p.m. of carotene or more are necessary for the maintenance of good levels of carotene in blood and milk. In the mow curing of baled hay, unheated air apparently cannot be depended upon regularly to produce a superior product.

P57 Stack Finishing of Baled Hay with and without Heat. K. A. KENDALL, W. B. NEVENS, AND J. H. RAMSER, University of Illinois.

Numerous trials in the finish curing of long, chopped, and baled hay by means of a mow ventilation system have shown this method of curing to be advantageous as compared with field curing. Six trials in the finish curing of baled hay were carried out, two of them with supplemental heat supplied by oil-burning units. Stacks ranging in size from 135 to 300 bales were constructed with a central duct formed by bales.

The moisture content of hay as baled should be no higher than for finish curing in other forms. Baled hay with 47 per cent moisture could not be dried even with supplemental heat. Hay baled with about 30 per cent moisture was successfully dried. Samples showed 15.7 per cent protein and 21,000 units carotene per pound (average values). Eleven gallons of fuel oil and two gallons of gasoline were used per ton of dry hay. Bales tightly tied could not be dried successfully. Small, loose bales are needed. All bales must be stacked on edge and those next the duct spaced 1 to 2 inches apart to permit circulation of air. The outside bales must be placed close together and tarpaulins kept tightly tied over the stack to aid air circulation. Supplemental heat permits drying at night and during rainy weather.

P58 Conservation of Nutrients and Feeding Value of Wilted Silage, Barn-cured Hay and a Poor Quality Field-cured Hay. J. B. SHEPHERD, L. G. SCHOENLEBER, H. G. WISEMAN, C. G. MELIN, W. J. SWEETMAN, W. H. HOSTERMAN, AND H. M. TYSDAL, Bureau of Dairy Industry; Bureau of Plant Industry, Soils, and Agricultural Engineering; of Agricultural Research Administration; and Hay Section, Grain Branch, Production and Marketing Administration. United States Department of Agriculture.

Cooperative forage harvesting and feeding experiments were conducted

at Beltsville for the third year during 1947. In contrast with previous years, unfavorable weather occurred during harvest and the field-cured hay was badly damaged by rain. Therefore, the field-cured hay was of poor quality while the silage and barn-cured hay were of good quality. The second cutting alfalfa crop used was variable and quite weedy. The silage and barn dried hays were harvested with a field chopper. Supplemental heat was used in barn hay drying.

Nutrient preservation in the wilted alfalfa silage, barn-dried hay and field-cured hay was, respectively: dry matter, 86, 91 and 60 per cent; protein, 83, 83 and 49 per cent; carotene, 3.6, 5.3 and 0.6 per cent. The labor and equipment requirements were about the same for the silage and barn-dried hay but were much higher for the field-cured hay due to large field losses.

Average daily milk production per cow on wilted silage, barn-dried hay and field-cured hay was 37.1, 36.2 and 35.2 lb., respectively, and the 30-day declines in production were 7.7, 8.8 and 13.6 per cent. On an acre basis (including other feeds), milk production was 40 per cent higher on the silage and 48 per cent higher on the barn-dried hay than on the field-cured hay.

P59 Vitamin D Content of Forages as Affected by Various Curing Procedures. J. W. THOMAS AND L. A. MOORE, Bureau of Dairy Industry, U. S. Department of Agriculture.

The vitamin D content, expressed in International Units per pound of air-dry material, of an alfalfa crop harvested in 1945 as wilted silage, barn-dried hay and field-cured hay, was 254, 213 and 440, respectively. Values of 393, 264 and 400, respectively, were obtained for alfalfa harvested in 1946.

Alfalfa brought into the barn for mow drying immediately after cutting and at three stages of maturity was found to contain appreciable amounts of vitamin D. The alfalfa cut at the mature or seed stage contained much more vitamin D than that cut at half-bloom. The latter contained slightly more than that cut at bud stage. All three hays supplied sufficient vitamin D to prevent any visible signs of rickets in calves when the hays were fed as the only source of vitamin D to calves kept in a darkened barn from birth to 8 months of age.

The amount of dead leaves adhering to the plant at cutting time of the bud, half-bloom and mature stages was 2.4, 2.9 and 6.5 per cent, respectively. These dead leaves contained approximately 3,200 I.U. of vitamin D per pound of air-dry material. No vitamin D was detected in totally green leaves. Thus, the amount of dead leaves adhering to the harvested forage plant may modify considerably the vitamin D content of forages irrespective of length of exposure to sunlight during curing.

P60 Comparison of Early-cut and Late-cut Lespedeza Hay for Milk Production. C. E. WYLIE, J. A. EWING, ERIC W. SWANSON, AND J. M. MADDUX, University of Tennessee.

The differences in feeding value of Korean lespedeza hay cut in the bloom stage and in full seed stage have been measured with milk cows in five winter feeding trials. In the first four trials, groups of six cows each were fed *ad libitum* early- and late-cut lespedeza hay, respectively. They also were fed 10 lb. of corn silage daily and concentrates according to production. The early-cut hay was more palatable, was consumed in larger quantities, and resulted in more milk production. The average production per ton of hay fed was 109 lb. 4 per cent fat-corrected milk more for the early-cut hay. The early-cut hay gave a greater return per ton in three of the four *ad libitum* trials. In the fifth trial, lespedeza hay was the sole roughage ration and grain was fed at a wider ratio to milk production. Carefully paired milking cows were placed in two groups of five and fed so that hay consumption was equal. The average daily production per cow was 3.58 lb. of 4 per cent milk higher on the early-cut hay, with a progressive increase in difference between the two groups as the trial continued. The average fortnightly decline was 0.50 lb. 4 per cent milk in the group fed early-cut hay and 0.72 lb. in the group fed late-cut hay.

P61 The Influence of Various Hays on the Production, Vitamin Content, and Flavor of Milk. J. K. LOOLSI, V. N. KRUKOVSKY, AND G. P. LOFGREEN, Cornell University.

Fifteen Holstein cows were fed six types of hay in an incomplete block design experiment involving four periods of 5 weeks each. The hays studied included early-cut timothy, late-cut timothy, second crop alfalfa cut at early and late stages of maturity, birdsfoot trifol (*Lotus corniculatus*) and ladino clover. Measurements were made of the palatability of the hays and of their effects upon milk production and on the carotene, vitamin A and tocopherol contents and flavor of the milk.

The late-cut timothy proved much less palatable and resulted in a lower milk production than any of the other hays. On *ad libitum* feeding the average intake of late-cut timothy was only 35 to 44 per cent as much as of the other hays, and the actual milk production was approximately 25 per cent lower.

The milk produced when birdsfoot trifol was fed was appreciably higher in carotene, vitamin A and tocopherol content than from any other hay. The milk produced during the periods when ladino clover and late-cut timothy were fed was lower in these vitamins than when early-cut timothy or alfalfa hay was fed. Milk of poor keeping quality resulted during ladino feeding and could be correlated with the low contents of vitamin A, carotene and tocopherol. The study will be repeated to obtain more infor-

mation concerning the influence of hay upon the fat soluble vitamin content and the keeping qualities of the milk.

P62 Comparison of Digestion Coefficients of Sun-cured and Barn-cured Hays from the Same Field. O. M. CAMBURN, Vermont Agricultural Experiment Station.

This report covers three crop years, 1944-1946, for which there were, respectively, two, four and three pairs of hays. From each field, after partial field curing, one portion was placed on flues in the barn to be finished. Another portion was sun cured and then stored to sweat-out in the mow.

Each year the barn-cured hay, as fed, carried more crude protein and nitrogen-free extract than the sun-cured hay which was higher in crude fiber content. The ether extract contents were similar. For barn-cured hay, the digestion coefficients were higher for crude protein for 2 years and higher every year for nitrogen-free extract. For sun-cured hay, the crude fiber coefficients were higher every year; ether extracts were higher for 2 years. The digestible protein was higher for barn-cured hay for 2 years and identical 1 year; for digestible nitrogen-free extract, the barn-cured hay excelled for 3 years; digestible crude fiber of the sun-cured hay was higher for 3 years and ether extract for 2 years. When the results for 3 years are averaged, the digestible protein and total digestible nutrients are similar for hays cured by these two methods.

P63 Lactating Factors for Dairy Cows in Dried Grapefruit Peel. R. N. DAVIS AND A. R. KEMMERER, University of Arizona.

Eight cows—four Guernseys, two Jerseys, and two Holsteins—were fed alfalfa hay *ad libitum* until milk production definitely decreased. At the end of this period the ration was supplemented daily with 4 lb. of dried grapefruit peel for 5 weeks. Then the dried citrus was replaced with an equal amount of a mixture consisting of rolled barley 6 parts, wheat bran 6, cottonseed meal 2, and beet pulp 2. After 4 weeks this ration was supplemented with oat pasture. Four pounds of dried grapefruit peel added daily to an alfalfa hay ration increased milk production. An equal amount of a grain mixture did not maintain this increase. Supplementing a ration of alfalfa hay and concentrate mixture with oat pasture definitely increased milk production. Dried grapefruit peel contains factors which stimulate milk production in dairy cows.

P65 The Growth of Dairy Heifers Reared on Maximum Roughage with Varying Amounts of Grain.¹ O. T. STALLCUP, H. A. HERMAN, AND A. C. RAGSDALE, University of Missouri.

Feed consumption and growth of Holstein heifers on rations utilizing

¹ Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station; Journal Series no. 1112.

hay, silage and roughage to a maximum extent, together with minimum amounts of grain, have been recorded. Three trials were conducted in which the average consumption of feedstuffs and pasture from 6 to 24 months of age was as follows: (a) 796 lb. grain, 1728 lb. lespedeza hay, 115 lb. alfalfa hay, 2265 lb. sorgo silage, and 334 days on pasture; (b) 912 lb. grain, 3934 lb. lespedeza hay, 3757 lb. sorgo silage, and 262 days on pasture; (c) 1232 lb. grain, 1870 lb. lespedeza hay, 773 lb. alfalfa hay, 1975 lb. mixed clover and grass hay, and 263 days on pasture.

The mean body weight of all groups was slightly below normal standards at 24 months of age. The height at withers was only slightly below normal for all groups. The circumference of chest was slightly above normal in the first group and slightly below normal in the other groups. A fourth group of heifers fed on hay, silage, pasture and minerals from 15 to 24 months of age was slightly below normal in weight at 24 months. None of the deviations of the groups from normal, either in body weight or in body measurements, was statistically significant. It is concluded that heifers may be grown satisfactorily from 6 to 24 months of age on rations utilizing hay, silage and pasture to the maximum with but little sacrifice in growth.

P66 Wintering Dairy Heifers on Legume Hay. S. A. HINTON, J. T. MILES, AND C. E. WYLIE, Tennessee Agricultural Experiment Station.

Results of trials at Knoxville show that dairy heifers over 1 year of age can be well grown without concentrate feeding if good pastures are provided in the spring, summer and fall and if ample amounts of legume hay are fed during the winter. The trials included comparisons of field-cured and air-dried hay as winter feed for heifers. Sixteen yearling dairy heifers pastured on permanent pasture through the summer and fall were brought into the barn on December 1. They were divided as equally as possible into two groups, one group being fed field-cured long hay and the other bin-dried chopped hay. No concentrates were fed. Salt and minerals were kept before them at all times.

Heifers fed both kinds of hay made normal gains, averaging 1.38 lb. per day per heifer for those on field-dried hay and 1.33 lb. when fed bin-dried hay. Heifers ate an average of 25.7 lb. each daily of the field-cured hay and 24.2 of the bin-dried hay. Holstein heifers made much greater gains on the hay feeding than did the Jerseys; however, when they were put on pasture after the hay feeding period, the Jerseys gained as rapidly as did the Holsteins. Heifers in both groups and of both breeds gained approximately 1 lb. each daily on pasture.

- P67 Observations on Calves Dehorned with an Antimony Trichloride-salicylic Acid-collodion Preparation. G. E. STODDARD, University of Wisconsin.

More than 60 calves have been dehorned with solutions containing antimony trichloride as the escharotic, salicylic acid as an analgesic, and flexible collodion as the carrier. Two solutions containing 28 and 38 per cent, respectively, of antimony trichloride, a commercial preparation containing an undisclosed amount of antimony trichloride, and stick caustic potash were compared. The 38 per cent solution, being more viscous, was easier to apply with a minimum of spreading. It also formed a firm film more rapidly, reducing the time required for application. The solution was applied by an eye-dropper with a spatula-shaped tip. The three solutions and caustic potash were about equally effective. The caustic potash was very irritating, whereas the calves dehorned with the antimony trichloride solutions showed no apparent pain during application. After about an hour, some exhibited a slight irritation, as shown by the shaking of their heads. The amounts required ranged from 1 to 2 g., with the smaller amounts used for the small horn buttons. The area covered should be kept to a minimum, covering only the button elevation. The amount necessary to remove the horn from some calves has been found to cause excessive swelling on others. The Guernsey calves seemed more resistant to escharotic action than did the Holstein, Ayrshire, Jersey and Brown Swiss calves.

The preparations were effective for calves between the ages of 3 and 10 days, but results were best at 3 to 5 days. Calves have been successfully dehorned at ages up to 17 days, but earlier treatment is recommended. The dehorning operation with the antimony trichloride solution took about twice as long as with caustic potash. Initial trials on dehorning kids have proved unsuccessful with all escharotics used.

- P68 Comparison of Various Methods of Cooling Dairy Cows in Summer. D. M. SEATH, AND G. D. MILLER, Kentucky Agricultural Experiment Station and Louisiana Agricultural Experiment Station.

Three experiments were conducted in an effort to determine the efficiency of various methods of cooling cows in summer months. During the three trials the air temperatures as taken during daytime experimental periods averaged 88, 92.6 and 90.7° F., respectively. Cows either were placed in the sun for 2 hours prior to treatment or two lots of cows were used so that one could remain in the sun and serve as a check while the other received the cooling treatment. The effects of cooling treatments were measured by changes in body temperature and respiration rate.

Air movement as produced by a fan and directed at cows under a shade

shelter was more efficient in cooling than was the shade alone. The sprinkling of cows with water at 85° F. tended to cool them faster than did the fan, but at the end of 1 hour there was only a little difference. Sprinkling when combined with a fan was more efficient than either the sprinkling or fan alone.

A self-serving sprinkling device kept cows cooler than any of the other methods. This device was constructed by using a series of fog-producing self-cleaning spray nozzles attached to 0.5-inch pipe and suspended from the ceiling of a home-made bamboo shelter. Water under approximately 40 lb. pressure produced a mist-like spray which cows liked and used freely on warm sunny days. As a result, the body temperatures of the experimental cows remained normal or even lower and respiration rates near normal on relatively warm days.

P69 Relation of Management to the Let-down of Milk. C. E. KNOOP, Ohio Agricultural Experiment Station.

Twelve cows in the peak of lactation and two cows in the latter part of lactation were milked with a specially-designed milking machine in order to study the effects of temperature of udder wash water upon the let-down of milk. All factors relating to preparation and milking of the cows were standard, except temperatures of the udder wash water, which were as follows: cold (50 and 64° F.), warm (100° F.), and hot (132° F.). Related factors such as the inheritance of cows to milk at slow, medium, or rapid rates; conditions such as seasons of the year (spring 45° F. and summer 80° F.); effects of additional oxytocin; and variation in milking machine vacuum were included in this study.

Let-down of milk as determined by the amounts of milk taken from cows during the first 1 to 1.5 minutes of milking time and length of the total milking period were not influenced by temperature of the udder wash water. Even though further work is necessary, the results indicate that cows milk slowly or rapidly depending upon the size of the orifice ducts, strength of the sphincter muscles, and management routine.

P70 The Effect of Time of Milking after Milk Excretion on Total Milk Production. GERALD M. WARD AND VEARL R. SMITH, University of Wisconsin.

The effect of milking cows at different intervals after milk excretion was ascertained on five cows in various stages of lactation and levels of production. The mean production of each half of the udder was determined during a preliminary period by milking each half of the udder simultaneously into a separate container. During the preliminary period, the cows were prepared for milking by a hot water massage and the milkers

were attached 2 minutes later. During the experimental periods, the half of the udder which served as a control was milked 2 minutes after preparation and the other half was milked at 4, 8, 12, 16, or 20 minutes after preparation. One half of the udder alternately served as control and experimental. The experimental periods were 5 days in length, followed by a 2-day transition period. Treatments were at random. The experimental period was followed by a 5-day post-experimental period. Milk production for either half of the udder was significantly less when that half of the udder was milked at 12, 16 and 20 minutes after preparation as compared to the control period of milking 2 minutes after preparation. Milk production with the 4- and 8-minute treatments was not significantly different from the control.

P71 Silage or Winter Pasture for Dairy Cows. C. E. WYLIE, S. A. HINTON, AND L. R. NEEL, Tennessee Experiment Station.

In order to compare winter grazing with the feeding of silage in maintaining dairy cows in the winter months, feeding trials were set up. Three groups of Jersey cows were used. Group I was fed corn silage, hay and grain. Group II was fed hay and grain and allowed to graze annual winter pasture. Group III was fed hay and grain and allowed to graze rye and crimson clover pasture. In addition, this group was allowed to run on bluegrass and white clover pasture when the annual pasture field was considered too soft for tramping.

Trials were continued for four annual winter periods of 150 days each. Each year the production of the cows in Group III exceeded that of those in Groups I and II. Group II produced more than Group I in three of the four feeding periods, while Group I exceeded II one winter. Cows were able to run on annual winter pasture an average of 64 days per winter and on permanent pasture an additional 65 days. Days unsuitable for cows to be outside the barn averaged 21 per season, varying from a minimum of 9 to a maximum of 40.

P72 Sweet Sudan Grass as a Forage Crop for Dairy Cattle. K. A. KENDALL AND W. B. NEVENS, University of Illinois.

An 80 per cent sweet Sudan grass and 20 per cent soybean mixture containing 25-30 per cent dry matter when ensiled without added preservatives produced ensilage of excellent quality. It contained 1.42 per cent acidity. Twenty cows fed corn silage and sweet Sudan-soybean silage in a pair-fed, double reversal feeding trial produced 18,092 lb. fat-corrected milk and 17,480 lb. fat-corrected milk, respectively. The dry matter of the two silages as fed was 29.6 per cent for corn and 26.5 per cent for the sweet Sudan-soybean silage. For pasture, a sweet Sudan-soybean mixture

was compared with a common Sudan grass-soybean mixture. The former was more palatable to dairy cattle and remained greener throughout the pasture season. Sweet Sudan grass was found more resistant to disease than the common variety. The average prussic acid content of the sweet Sudan and common Sudan grass was 0.00204 per cent and 0.00216 per cent, respectively, when the crops were 18-30 inches in height.

P73 Pastures in Relation to Dairy Development in the South. R. H. LUSH, Tennessee Experiment Station.

The South has 25 per cent of the milk cows on 34 per cent of the farm land and produces only 17.8 per cent of the milk in the U. S. In 1944, over 40,000 less farms reported milk cows than in 1939, but the production per farm has increased about 1,000 lb. However, 80,932 more farmers, an increase of 47 per cent, now are selling whole milk off their farms. The dairy farms of the South are becoming fewer in number but larger in production than in the country as a whole. A continued shift in that direction calls for more uniform year-around production than where cream or home consumption was the chief outlet. Results obtained a few years ago at both the Middle and West Tennessee Experiment Stations favored nearly year-around grazing with little grain feeding. Recent information from the various Southern states has emphasized that where annual grazing crops are used, an early date of planting with liberal seeding and fertilizing of well-prepared land is essential for milk production. Improved practices involving the use of Sudan grass, millet, lespedeza and alfalfa-grass mixtures have helped even summer production. Old bluegrass pasture alone is not dependable but needs improving in two or more ways to be really productive.

P74 Irrigated Pastures for Dairy Cows. JOHN A. EWING, N. MADDUX, C. E. WYLIE, AND R. H. LUSH,¹ Middle Tennessee Experiment Station.

Results of irrigating permanent pastures to provide summer grazing are presented for 1945-1947. A bluegrass pasture of 13.2 acres was divided into nearly equal areas in 1945 and the same area irrigated by means of a sprinkling system, depending on moisture conditions. Two groups of Jersey cows, fed 2 lb. grain each daily and with access to alfalfa hay in racks, were used and numbers adjusted according to grazing available. Slightly more hay was eaten each year by the cows on non-irrigated pasture, but there was an average of 19 lb. greater gain in liveweight by cows on irrigated pasture. The average for three years, 1945-1947, shows cows on

¹ Engineers of the Tennessee Valley Authority. J. K. Underwood, Agronomist, and in the early tests, L. R. Neel, former Superintendent, cooperated.

irrigated pasture obtained 33 per cent or 47 more cow-days of grazing, produced 35 per cent or 1,193 lb. more milk, and returned \$39.85 or 27 per cent more above feed and irrigation costs per acre than did the cows on non-irrigated pasture. With normal depreciation costs of irrigation equipment included in the above figures, irrigation appears very profitable at present prices or where an adequate cheap water supply exists, and for maintaining summer milk production.

P75 Increasing the Production of Permanent Pastures through Renovation. J. B. SHEPHERD, R. E. WAGNER, R. E. HODGSON, W. J. SWEETMAN, AND C. G. MELIN, Bureau of Dairy Industry, and Bureau of Plant Industry, Soils and Agricultural Engineering, U. S. Department of Agriculture.

At Beltsville, permanent pastures have shown an increase in grazing capacity of 16 per cent due to fertilization and a further increase of 10 per cent due to rotation grazing. Additional improvement has been made through renovation consisting of tillage and seeding to high yielding grasses and legumes. One Kentucky bluegrass pasture and one orchard grass-bluegrass pasture were renovated in the spring of 1945 and another of each in the spring of 1946. Renovated and check pastures were lined, manured, and fertilized alike. All pastures were rotation grazed. The year of renovation, a light first-cutting was harvested for silage and the pastures grazed afterwards. For 1945 to 1947, inclusive, the production of the pastures from grazing, in terms of standard grazing days (16 lb. T.D.N.) and good hay equivalent were, respectively: Kentucky bluegrass pasture check 177 days and 5,662 lb.; first year after renovation, 115 days and 3,892 lb. or 69 per cent; second year, 276 days and 8,828 lb. or 156 per cent; third year, 257 days and 8,214 lb. or 145 per cent. Orchard grass-bluegrass pasture check 174 days and 5,562 lb.; first year after renovation, 130 days and 4,159 lb. or 75 per cent; second year, 243 days and 7,788 lb. or 140 per cent; third year, 244 days and 7,810 lb. or 140 per cent. Averaging all years and all pastures, renovation increased grazing over fertilized, rotation-grazed permanent pastures by 21 per cent.

P76 Effect of Intermittent and Limited Winter Grazing of Rye Pasture on the Carotene and Vitamin A Content of Cows' Milk. R. G. WASHBURN AND C. F. MONROE, Ohio Agricultural Experiment Station.

Two groups of cows were fed a ration of corn silage, alfalfa hay and grain. Beginning in November, 1947, one of these groups was barn fed on this ration throughout the experiment, while the other group, in addition to this ration, was allowed to graze rye pasture for a brief period on

days when the weather was suitable. Due to the severity of the winter, the cows in the grazing group were not pastured as much as had been expected, but these cows ate well of the rye.

The carotene and vitamin A content of the milk in the preliminary period was 1,668 U.S.P. units vitamin A activity per l. for the control group and 1,778 units per l. for the pasture group. By November 25, cows in the control group had decreased to 1,382 units per l., while the group which had received 95 hours of pasture during this period had increased to 2,343 units per l. In the last week of January, 1948, the control group had further decreased to 1,009 units per l. and the other group which received only 17 hours on pasture had decreased to 1,176 units per l.

MANUFACTURING SECTION

- M1 The Effect of the Addition of Ascorbic Acid to Milk on the Keeping Quality of Its Dried Product. GEORGE R. GREENBANK AND PHILIP A. WRIGHT, Bureau of Dairy Industry, U. S. Department of Agriculture.

Four different groups of samples of dried whole milk were prepared from milks containing differing amounts of added ascorbic acid. In each group the keeping quality of samples containing different concentrations of ascorbic acid was compared with a control which was made from the same milk but which contained no added ascorbic acid. In every case the control had the poorest keeping quality and the keeping quality of the other samples of the group increased as the amount of added ascorbic acid was increased. It was observed that the apparent ascorbic acid content (all substances which reduce 2-6 dichlorophenolindophenol) decreased rapidly at first but that later the apparent ascorbic acid increased. In samples of the best keeping quality the decrease was not great and eventually the concentration of apparent ascorbic acid became greater than the initial ascorbic acid concentration. From these observations it may be concluded that ascorbic acid protects the fat against oxidation. When sufficient ascorbic acid or reducing substances are present to protect the fat from oxidation until the dried milk generates reducing substances faster than they are destroyed in storage, prolonged keeping quality will result.

- M2 The Formation and Preservation of Antioxidants by Special Methods of Processing in the Preparation of Dried Milk. GEORGE R. GREENBANK AND PHILIP A. WRIGHT, Bureau of Dairy Industry, U. S. Department of Agriculture.

High heat treatment of milk reduces its redox potential (E_h) and improves the keeping quality of its dried product. The decrease in E_h depends on the conditions under which the milk is heated. Heating in an

open pan causes the least decrease in E_h . The same heat treatment in a full can, out of contact with air, which simulates that obtained with a tubular heater, causes approximately twice the decrease in E_h found by heating in an open pan. Heating deaerated milk for the same time and temperature in a full can causes approximately three times the decrease in E_h observed in open pan heating, indicating that there is a higher concentration of sulphhydryl groups, which are considered to be antioxidants.

Thirty samples of dried milk were prepared from normal and deaerated milk to determine the effect of the heat treatment of deaerated milk on the keeping quality of its dried product. The control sample of each pair was heated in a hotwell to the same temperature and for the same time as the deaerated sample. Twenty-seven of the pairs showed that deaeration before heat treatment improved the keeping quality of the dried product. These data indicate that heating deaerated milk preserves the substances which protect the fat more than the same heat treatment in contact with air.

M3 The Effect of Heat Treatment on the Reducing Systems of Milk.

S. T. COULTER, HERBERT HARLAND, AND ROBERT JENNESS, University of Minnesota.

Two methods have been used to determine the reducing capacity of fluid milk and dry whole milk—the thiamine disulfide method of Harland and Ashworth and the acid ferricyanide method of Chapman and McFarlane as modified in this laboratory. Based on work with simplified systems, the thiamine disulfide method measures essentially only those reducing groups produced as a result of heat treatment of the serum protein fraction of milk. The acid ferricyanide method includes these groups as well as ascorbic acid and reducing materials resulting from heat treatment of lactose in the presence of phosphate buffer or protein or both.

Data have been secured showing that the thiamine disulfide reducing substances reach a maximum during heat treatment of fluid milk and then decrease. The maximum reached is somewhat higher and the rate of decrease is slower in the absence of oxygen. A higher maximum is reached by high-temperature short-time heating (95° C. for 2 to 5 minutes) than by heating to a lower temperature for a longer time. The acid ferricyanide reducing groups, although showing some effect of oxidation during heating, continue to increase over long periods of heating.

The acid ferricyanide reducing capacity of dry whole milk may be increased as a result of heat treatment during drying. The thiamine disulfide reducing groups cannot be increased by the drying process. As a matter of fact, there is no change in the thiamine disulfide reducing capacity on heating systems which have a higher solids content than about 70 per cent. The maximum rate of production of acid ferricyanide reducing substances occurs on the heating of milk systems of about 90 per cent solids.

M3-a The Heat Treatment of Milk Necessary to Prevent Lipolytic Action in Its Dried Product (A Preliminary Report). GEORGE R. GREENBANK AND PHILIP A. WRIGHT, Bureau of Dairy Industry, U. S. Department of Agriculture.

The heat treatment of milk used in making dried milk must be at least sufficient to prevent lipolytic activity. The time and temperature required to do this is not known. In certain samples of dried whole milk prepared from milk heated in a hotwell to high temperatures, the lipase still was active. Samples prepared from milks that had been heated for 30 minutes at 142, 152 or 162° F. (61.1, 66.7 or 72.2° C.), respectively, developed rancidity within 112, 126 and 140 days, respectively, when stored at 86° F. (30° C.). These data indicate that the lipase in milk is more resistant to heat treatment than commonly is supposed. Work is in progress on the destruction of lipase by short-time high-temperature heat treatment.

M4 The Isolation of Compounds Responsible for the Stale Flavor Developed in Dried Whole Milk. I. The Distribution of Stale Flavor between the Fraction of Reconstituted Stale Whole Milk Powder.¹ R. McL. WHITNEY AND P. H. TRACY, University of Illinois.

This study was undertaken in the belief that, with the isolation and identification of the chemical compounds responsible for the stale flavor which develops in dried whole milk, a better knowledge of the mechanism of its formation could be obtained and preventive measures developed. Dried whole milk was prepared and stored under varied conditions to obtain a continuous supply of stale powder. Reconstituted stale dehydrated milk was separated into cream and skim milk. The cream was churned into butter and buttermilk, or washed until it "oiled-off." The butter and the washed cream were melted at 40° C., centrifuged, and filtered to yield butter oil and butter serum. The various fractions so obtained were reconstituted with appropriate fresh products to the composition of the original milk. These reconstituted milks were blended in concentration series with fresh whole milk and submitted to a judging panel to determine the threshold concentration of the stale fraction.

In all determinations made on the whole milk, cream, washed cream, butter and butter oil, the stale flavor component appeared to distribute itself according to the amount of fat present in the stale fraction. There-

¹ This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned no. 181 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of the Army.

fore, it is indicated that the stale flavor component is concentrated in the butter oil.

M5 A Solubility Method for the Determination of Alpha and Beta Lactose in Dry Products of Milk. R. P. CHOI, C. W. TATTER, AND B. W. FAIRBANKS, American Dry Milk Institute, Inc., Chicago, Illinois.

Based upon the maximum rate of solution of lactose hydrate and upon the difference in initial solubilities of the *alpha* hydrate and the *beta* anhydride, a simple method has been developed for the determination of the two forms of lactose in dry products of milk. The essential steps consist of adding an excess of lactose hydrate to a known quantity of the sample, determining the solubility at several time intervals, and extrapolating to zero time to obtain the total initial solubility. The *beta* lactose content may be calculated easily from the fact that within a certain range of *beta* concentration the total initial solubility is the sum of the initial solubility of the *alpha* hydrate and of the quantity of *beta* lactose present. The *alpha* modification may be ascertained by determining the total lactose of the sample. Results for nonfat dry milk solids and dry whey solids by this method are in good agreement with those reported in the literature.

M6 The Viscosity and Heat Stability of Concentrated Milks Subjected to High Temperature Processing. B. H. WEBB AND C. F. HUFNAGEL, Bureau of Dairy Industry, U. S. Department of Agriculture.

The relative viscosity and heat stability attained by concentrated milks during their manufacture into various condensed or dried products is a factor in determining the amount of heat that can be applied and the physical characteristics of the finished product. Some of the relationships between the concentration, viscosity and heat stability of skim milks heated by different methods to temperatures between 160 and 280° F. have been studied. Skim milks of 23 to 28 per cent solids have sufficient heat stability to enable them to withstand a sterilization heat treatment after canning or before drying. At higher solids concentrations, coagulation occurs before the product can be sterilized. Heat treatments intermediate between pasteurization and sterilization were applied to skim milks of concentrations up to 45 per cent solids. Agitation during heating causes visible coagulation to occur sooner than when the milk is heated with a minimum of agitation. Continued agitation or pumping during the onset of coagulation drastically reduces the thickening that normally accompanies the formation of a visible coagulum.

- M7 The Microbiological Keeping Quality of Bulk Condensed Milk. A. M. PEARSON, Ontario Agricultural College, AND F. E. NELSON, Iowa State College.

Samples of bulk condensed milk obtained from several plants at different times were held at 38, 44, 55, and, in some cases, 70° F. Changes in organoleptic characteristics, standard plate count and coliform count were followed at appropriate intervals, using a separate 2-oz. bottle of sample at each time interval. Titratable acidity, pH and pasteurization efficiency in a standardized ice cream mix were followed at the same intervals on four of the samples. Storage at 70° F. resulted in defect appearance within 2 days for nearly all samples. As the storage temperature was lowered, defects required considerably longer times to appear, only one sample becoming organoleptically unsatisfactory after 8 days at 38° F. Bacterial counts, acidity and pH became unsatisfactory somewhat before the samples were rejected for off-flavors. The lower the initial plate count or coliform count, usually the longer the samples remained satisfactory at any given storage temperature. When condensed milk from the same original lot was used, the count on the pasteurized mix in which it was incorporated largely was independent of the time and temperature, within the limits of this experiment, at which the condensed milk had been stored.

- M8 The Use of Sweetened Condensed Whole Milk in the Manufacture of Caramels. J. J. SHEURING AND P. H. TRACY, Illinois Agricultural Experiment Station.

The quality and processing treatment of sweetened condensed whole milk should influence the color, body, texture and flavor of caramels into which it is manufactured, since the caramels consist of approximately 50 per cent of sweetened condensed whole milk. This study was undertaken to investigate the factors of forewarming temperatures and times, emulsifiers, composition, bacterial quality, salt balance and flavor of whole milk upon the sweetened condensed whole milk and the caramels into which it was manufactured. All the sweetened condensed whole milk was manufactured in the College creamery. Caramels were made in the kitchen of a candy company using commercial procedures.

Forewarming temperatures of at least 180° F. for 5 minutes for the milk were found to be desirable, as evidenced by light color, good flavor, shortness of texture, and firmness of body of the caramels. Seasonal variation of milk had no significant effect upon the flavor or body of the caramels. The addition of 0.075 per cent sodium citrate to the milk resulted in soft caramels, while the same amount of calcium oxide caused caramels to be too hard for cutting properly. The addition of 1½ ounces per 10 lb.

of fat sorbitan monostearate, and a mannitol derivative of beef fat improved the body of caramels, while an equal amount of soybean lecithin resulted in a sticky caramel that did not wrap efficiently.

M9 Influence of the Mineral Content of Water on the Properties of Ice Cream Mixes. ROBERT A. HIBBS AND W. A. KRIENKE, Florida Agricultural Experiment Station.

Several series of ice cream mixes, of average composition, were prepared from high fat content cream and plain condensed skim milk as the only sources of milk constituents, sucrose the only sugar, sodium alginate the stabilizer and water of varying degrees of hardness the diluent for completing the mixes. Distilled water was the diluent used in the control mixes. Processing procedures of the mixes were varied to include the homogenization of some complete mixes and of certain fractions of other mixes that consisted of the cream and part or all of the plain condensed skim milk with or without some of the water.

Effects of the minerals contributed by the hard water usually were reflected in increased clumping of the fat, retarded whipping of the mixes, reduced body and texture scores of the ice cream and increased curdy appearance of the melting ice cream. The most desirable mixes in this study had been homogenized as complete mixes.

M10 Observations on the Effects of Various Stabilizing and Emulsifying Materials on the Properties of Ice Cream. W. S. ARBUCKLE, R. B. REDFERN, AND L. F. BLANTON, North Carolina State College.

A comparison study has been made of common commercial ice cream mix stabilizers and emulsifiers. The study included stabilizing products, products containing a combination of stabilizing and emulsifying agents and stabilizing products plus emulsifying products. Data showing the effect of stabilizing and emulsifying materials on the properties of the mix and the finished ice cream have been secured.

Results indicate that some stabilizers affected the acidity and pH value of the mix. Viscosity measurements show that certain stabilizers produced an increase in the viscosity during the processing procedure, with no further change during an aging period. Some stabilizers caused an increase in viscosity of the mix during the aging period as well as during the processing procedure, and other stabilizers produced an increase in viscosity only during the aging period.

In comparison with stabilizers, the products containing a combination of stabilizing and emulsifying agents showed a more uniform acidity and pH value, somewhat lower surface tension value and greater viscosity of the mix, and a slower rate of melting of the ice cream. Emulsifying agents

had little effect on the acidity and pH value of the mix, produced a less viscous mix with a lower surface tension value, and caused a slower rate of melting in the finished ice cream.

M11 The Effect of a Mannitol of Beef Fat on the Whipping Qualities, Body and Texture of Ice Cream. RALPH NADEN, J. J. SHEURING, AND P. H. TRACY, University of Illinois.

A group of chemically prepared substances known to the trade as "emulsifiers" or "whipping agents" are being used in the ice cream industry. The use of mono- and di-glycerides of both plant and animal origin as emulsifying agents in ice cream has been patented. The inventors claim that these products impart faster whipping and improve the body and texture of ice cream.

The mannitol derivative of beef fat (MBF) used in this study was arbitrarily selected, as preliminary studies have shown that it had a satisfactory effect upon the physical properties of ice cream. The addition of MBF improved the whipping of mixes containing more than 6 per cent milk fat. MBF had practically no effect upon the viscosities of ice cream mixes. The use of 0.2 per cent MBF, when used in combination with commonly used stabilizers, improved the whipping qualities of ice cream mixes. Dehydration of mixes retarded the beneficial effects of MBF. Shrinkage of ice creams containing MBF exceeded control samples in all cases. MBF improved the whipping qualities of ice cream made from neutralized cream, butter or frozen cream. Aging of the mixes was found to be less necessary when MBF was used. MBF decreased the whipping time of unhomogenized mixes.

M12 A Study of the Fat Emulsion in Natural and Artificial Creams. WALTER E. SNYDER AND H. H. SOMMER, University of Wisconsin.

An extensive study of the fat emulsion in natural and artificial creams was made with particular emphasis on the phenomenon of "fat clustering" and the physical and chemical changes occurring during a slow warming and cooling treatment herein termed a "rebodying process". The chemical composition of the fat globule "membrane", the viscosity and whipping of cream, the reaction of creams to temperature treatments, the surface tension phenomena of milk and cream, and the response of milks and creams, natural and artificial, to additions and subtractions of various constituents all were utilized in this study.

Conclusive evidence is given to support the view that lecithin, agglutinin, colostrum, euglobulin of milk, and euglobulin of colostrum all enhance the rebodding of cream. The composition of the membrane material of liquid globules differs from that of solid globules. Fat concentration,

lecithin concentration, salt composition, agglutinin, fat composition, lipolysis, average size and disparity in size of fat globules, and breed of cow were found to be important factors in the degree of rebodding response and whipping properties of natural and artificial creams. Marked surface tension changes were observed during the warming and cooling of milks and creams. Any correlation between agglutinin and surface tension values was not conclusively observed. Some evidence has been presented for the formation of a lipid-protein complex at the surface of artificially formed fat globules utilizing lecithin-butter oil-egg albumin and lecithin-butter oil-skim milk.

M13 Preservation of Milk for the Phosphatase Test. GEORGE P. SANDERS AND OSCAR S. SAGER, Bureau of Dairy Industry, U. S. Department of Agriculture.

Experiments with preservatives showed that the following, arranged in increasing order of their destructive effects on milk phosphatase, will preserve fresh milk for 2 to 3 weeks at room temperature: 1.5 per cent of chloroform; 3.5 per cent of toluene; 2 per cent of borax; 0.1 per cent of formaldehyde; 0.05 per cent of mercuric chloride; and 0.15 per cent of hydrogen peroxide. The last three reduced the enzymic activity rapidly. Borax interfered in the test, yielding milk with a pH of about 9.7 instead of the optimal pH of 10. Chloroform was more effective than toluene as a preservative, although neither inhibited the enzyme appreciably. Other advantages of chloroform are that its presence can be detected easily by its odor, and it prevents clumping of the fat.

Phenolic compounds, such as cresols, naphthols and salicylates, should not be used because they react with BQC and produce a blue color. It is recommended that 1.5 to 2 per cent of chloroform be used to preserve fluid products for this test. For solid products, chloroform—*e.g.*, 1.5 to 3 ml. in a 100-ml. container—can be put on a wad of cotton and placed with the sample in the container.

M14 Differentiation of Microbial Phosphatases from Milk Phosphatase. RALPH P. TITSLER, OSCAR S. SAGER, AND GEORGE P. SANDERS, Bureau of Dairy Industry, U. S. Department of Agriculture.

Approximately 200 strains of microorganisms, representing 90 species and 23 genera, were tested for production of phosphatase in milk and other media. Positive tests at pH 10 were obtained only with *Aerobacter*, *Escherichia*, *Pseudomonas*, *Bacillus*, *Lactobacillus enzymothermophilus*, *Penicillium camemberti*, *Penicillium roqueforti*, *Aspergillus niger*, and *Aspergillus oryzae*. Many of these in milk gave negative tests. All strains of *Streptococcus*, *Leuconostoc*, *Lactobacillus* (except one), *Propionibacterium*,

Bacterium linens, *Alcaligenes faecalis*, *Oidium lactis* and yeasts gave negative tests at pH 10.

Microbial phosphatases in dairy products ("false positive" tests) can be distinguished from milk phosphatase by two methods: The former generally are not inactivated appreciably at 70° C. for 5 minutes, while the milk enzyme is destroyed completely at 70° C. for 1.5 minutes; the few microorganisms that produce an alkaline phosphatase, active at pH 10, generally produce also an acid phosphatase, active at pH 4-6, while milk phosphatase is not active below pH 8. The order of thermal resistance of phosphatases was: coliforms and *Pseudomonas* (> 90° C. for 5 minutes); spore-formers (> 70° to > 80° C.); *P. roqueforti* and *A. niger* (70° C.); and *P. camemberti* and *A. oryzae* (< 70° C.). The last two can be distinguished by their acid phosphatase.

M15 A Solution for Time and Temperature Relationships for Inactivating the Phosphatase Enzyme in Milk. J. H. HETRICK, Dean Milk Co., AND P. H. TRACY, University of Illinois.

A small tube heat exchanger built on Mallory principles was used to process the milk. A calculated time of 0.83 second was required to heat to holding temperature. After holding for the desired length of time, the samples were rapidly cooled in ice water. The Sanders and Sager test (J. Dairy Sci. 29: 507. 1946) was used to determine the phosphatase activity, and a value of 1 unit per ml. of milk was used as the standard for satisfactory inactivation of the enzyme.

A semi-logarithmic relationship between temperature and time over the temperature range of 143-185° F. was found to exist for the thermal destruction of phosphatase. This relationship can be expressed by the following formula: $T = 174 - 9 \log t$ where T is the temperature (° F.) and t is the holding time (seconds) required to inactivate the enzyme at temperature (T). From this formula another expression was developed which indicates the destructive effect of each second of holding at any temperature (T) in reducing the phosphatase activity to a phenol equivalent of 1 unit per ml. of milk. This expression is $D = \text{Antilog } 10 \frac{(T-174)}{9}$. The summation of the D values multiplied by the time in seconds at the corresponding temperature must be 1 or greater for any time-temperature cycle to satisfactorily inactivate the phosphatase. Data secured with various heating rates indicate that the mathematical solution is satisfactory for practical use in determining time-temperature relationships necessary to give a negative phosphatase test in milk with various heating methods. It follows that if phosphatase inactivation is used as the standard for adequate pasteurization, this mathematical method could be applied to determine proper time-temperature conditions.

M16 Isolation of Heat-induced Flavor Compounds from Milk. STUART PATTON AND DONALD V. JOSEPHSON, Ohio State University.

Heat-induced flavor compounds have been removed from skim milk, heated to 260° F. for 60 minutes, by direct extraction with redistilled ethyl ether. These substances may be concentrated by evaporation of the ether. The judgments of many impartial observers indicate that this concentrate has a strong "caramelized" odor, typical of heated milk. Fractionation of the flavor concentrate has been accomplished by selective solvent extraction and vacuum distillation. Solvent extraction has yielded three major fractions soluble in water, petroleum ether, and ether, respectively. To date only the first and third fractions have been subjected to further investigation. The ether soluble fraction contains the "caramelized" principle, whereas the water soluble fraction has more of a sulfide-type of "cooked" odor. Both of these fractions contain reduced sulfur in significant quantities. This evidence may explain why the sulphydryl groups in heated milk disappear as the development of caramelized flavor progresses. Repeated tests for nitrogen on both of these fractions have been negative. Portions of the residue of the ether soluble fraction are vacuum distillable, but the principal "caramelized" substance, a yellow, sulfur-containing oil, could not be vacuum distilled under the conditions of the experiment.

M17 Some Observations on the Efficiency of High-temperature Short-time Pasteurization of Chocolate Milk. MARVIN L. SPECK, CHARLES D. COLVARD AND M. LEE SHUMAKER, North Carolina State College.

The heat treatment required for 99.99 per cent destruction of *Micrococcus freudenreichii* (no. MS66) in non-settling chocolate milk of different compositions and in whole milk was determined in laboratory pasteurization experiments using the technique previously described by Speck (J. Dairy Sci., 30: 975-981. 1947). The amounts of stabilizer (7.95-19.87 centipoise), sugar (5 and 8 per cent) and added nonfat dry milk solids (0 and 3 per cent) were varied in the chocolate milk studied. Differences in the per cent of these ingredients, as used in this study, had no appreciable effect on the heat treatment required to destroy the test culture. In comparing the resistance of *M. freudenreichii* in whole and in chocolate milk of the different compositions, no differences were observed at 165, 160, and 155° F. At 150, 145, and 143° F. longer exposures were required to destroy the test culture in chocolate milk than in whole milk. The adequacy of the standard for the holder method of pasteurization of chocolate milk, as contained in the Milk Ordinance and Code of the U. S. Public Health Service, 1939, therefore is questionable.

Preliminary experiments using a commercial HTST plate pasteurizer

have shown that temperatures of 161, 168, and 175° F. for 19 and 40 seconds gave reductions in the total bacterial count of chocolate milk comparable to that obtained by pasteurization at 145° F. for 30 minutes. The results have indicated that the final selection of a time and temperature for HTST pasteurization of chocolate milk may be as dependent upon the physical properties obtained on the product as on the minimum times and temperatures required to give satisfactory reduction in bacterial counts.

M18 Use of the Direct Microscopic Method for Pasteurized Dairy Products. M. J. PRUCHA AND VIRGINIA FRAZEE, University of Illinois.

The work was done in the Champaign-Urbana Public Health District laboratory during the last 2 years of the war. The community is operating under the United States Public Health Grade A milk ordinance. Samples of the pasteurized milk and cream were taken weekly from each milk plant and were examined by the standard plate method and also by the direct microscopic method. The purpose of the work was to determine whether the samples complied with the standard of the ordinance and not how closely the two methods agreed as to the exact count of bacteria in each sample.

In general, the two methods agreed on about 75 per cent of the samples as to whether the sample complied with the ordinance or not. Neither method alone gave complete information on the bacterial condition of the milk. In some cases, the milk was heavily loaded with bacteria but the plate method did not detect it. Sometimes the milk was contaminated subsequent to pasteurization and the direct microscopic method did not detect it.

M19 Bacteriophage Production by Cultures of *Streptococcus lactis*. F. J. BABEL, Purdue University.

Studies were conducted to determine the relationship between the number of *Streptococcus lactis* organisms used to inoculate given lots of milk and the bacteriophage titers of the milks, when the initial inoculations with bacteriophage were kept constant. As the amount of inoculum was decreased from 1.0 to 0.001 per cent, the final concentration of bacteriophage produced in the milks decreased. In these trials, secondary growth was most rapid in the cultures receiving the greatest inoculum and slowest in the culture receiving the smallest inoculum. A rather constant number of bacteriophage were produced from the bacterial cells added as the original inoculum when comparisons were made with the same culture and bacteriophage preparation. Two cultures of *S. lactis* which were sensitive to the same bacteria-free filtrate but which showed considerable difference in the time at which

secondary growth appeared were compared for bacteriophage production. One culture showed appreciable secondary growth in 6 hours, while the other culture was unusual because it was completely destroyed by bacteriophage and no secondary growth resulted. Both cultures produced approximately the same quantity of bacteriophage when the same number of organisms of each culture was added to milks inoculated with the same quantity of bacteria-free filtrate.

Several lots of milk inoculated with the same quantity of an *S. lactis* culture but with varying amounts of a bacteria-free filtrate active against the culture produced the same final bacteriophage titer. In these trials the initial bacteriophage titer could be varied from 10^{-1} to 10^{-7} and yet the final bacteriophage titer of the milks remained the same.

M20 Electron Microscope Studies of Bacteriophages Active against *Streptococcus lactis*. C. E. PARMELEE, P. H. CARR, AND F. E. NELSON, Iowa Agricultural Experiment Station.

Electron photomicrographs of bacteriophage particles active against *Streptococcus lactis* are presented. The photomicrographs of the cell-free bacteriophage particles were made from mounts on collodion membranes of a 1 to 100 water dilution of a cell-free whey filtrate having a titer of 10^{10} bacteriophage particles per milliliter. The photomicrographs of phage particles in the presence of bacterial cells were made from mounts prepared from mixtures of cell-free whey filtrates of bacteriophage and 24-hour cultures of susceptible *Streptococcus lactis* cells in 5 per cent whey solution. The mounts were dried for 12 to 14 hours, immersed in distilled water for 30 minutes, and again dried.

The mounts were prepared for observation and photography by shadowing with gold or platinum in a vacuum at an angle of about 75° . The bacteriophage particles are sperm-shaped and range from 0.18 to 0.28 μ in total length. The spherical head of the sperm-shaped particle appears to be 0.06 to 0.09 μ in diameter; the tail is 0.02 to 0.04 μ wide and 0.12 to 0.19 μ long.

M21 Some Factors Affecting the Rate of Acid Production by Cheese Cultures. H. C. OLSON AND FRANCIS D. COHENOUR, Oklahoma A. and M. College.

Various factors concerned with the daily propagation which might affect the rate of acid production by cheese cultures were studied. The factors which appeared to be of practical importance in increasing the rate of acid production by cheese cultures were increasing the solids-not-fat content of the milk, incubating at 70° F. rather than at 80 or 90° F., incubating until the cultures were thoroughly ripe, using enough inoculum to in-

sure a thoroughly ripe culture, and using freshly ripened cultures for inoculation of cheese milk.

The factors which appeared to have little influence on the rates of acid production by cheese cultures were: fat content of the milk used for daily propagation, bacterial content of the milk, temperature of heating of the milk above 165° F. for 30 minutes, and period of heating at 205° F.

M22 Methods of Controlling the pH of Fermenting Dairy Products and the Effects of pH Control. WAYNE I. TRETSVEN, Chicago, Illinois.

The hydrogen-ion concentration of dairy products is in part a function of chemical changes and directly affects the ensuing chemical changes as well as the physical qualities. In the manufacturing and handling of more or less concentrated, unsterile dairy products, such factors as temperature, light, and composition (concentration of moisture, sugar, salts, oxygen, etc.) have been the primary means of effecting the resulting changes.

The pH of cheese was changed by introducing desired gases. Cheese in carbon dioxide was found to ripen slightly slower than in nitrogen or *in vacuo* and much slower than when the pH had been increased by ammonia. A commercially produced fresh Cheddar cheese subjected to hydrogen chloride to reduce the pH to 4.2 failed to show any ripening in 15 months. The body and texture, color, and flavor were affected by the pH and could be controlled in part by pH.

Factors involved in changing the pH of cheese and other foods are size and shape, texture, moisture content, pH, time and concentration of reacting gas, variations in pressure, and temperature. By utilizing the procedures for altering and controlling the pH, new techniques in manufacturing and packaging have been found.

M23 Chemical Changes Occurring in Limburger Cheese during Accelerated Ripening. W. K. STONE AND S. L. TUCKEY, University of Illinois.

M24 A Preliminary Note on the Pasteurization of American Cheddar Cheese by Radio-frequency Heat. F. V. KOSIKOWSKY, B. L. HERRINGTON, AND A. C. DAHLBERG, Cornell University.

A number of batches of raw milk were made into American Cheddar cheese, which then was pressed overnight in Wilson square hoops. The pH of these cheeses varied from 5.1 to 5.3. The cheeses then were cut up into blocks (1.5 × 4 × 5.25"), packaged in Parakote, and heated directly by placing between the two electrodes of an experimental R.C.A. radio-frequency oscillator. The oscillator had a possible power output of 750 watts at 150

megacycles. The time required for heating 1.3-lb. packages of cheese to the desired temperature ranged from 1.5 to 2.7 minutes. Temperatures to which cheese were heated ranged from 117 to 155° F. After attaining the desired temperature, the cheeses were placed in cardboard boxes, air cooled, or held for 15 seconds and then air cooled at 70° F.

Raw milk cheeses held for 2 days at 50° F. and heated to as high as 146° F. retained their physical form. There was no oiling-off, and after cooling no noticeable difference was apparent between the heated and unheated samples. As the raw milk cheese became older, lower heating temperatures were required to obtain a desirable physical form.

Heating of most 2-day-old cheeses to temperatures as low as 130° F. with radio-frequency heat followed by air cooling at 70° F. destroyed on the order of 99.9 per cent of the bacteria and produced a phosphatase-negative cheese.

After ripening at 60° F. for 2 months, cheeses heated by radio-frequency showed definite signs of curing, in some cases closely approaching that of the raw control samples. Curing was indicated by increases in soluble protein, fatty acids, bacteria, and by activity of the decarboxylase enzyme system as determined by tyramine increases and finally by body breakdown and increase in flavor intensity. Some very high scoring cheeses were produced using radio-frequency heating, although extensive overheating produced oily flavors in some cheeses, while in others the cheese did not cure fully.

M25 Increasing Efficiency and Reducing Costs in the Manufacture of Cheddar Cheese. D. M. IRVINE AND W. V. PRICE, University of Wisconsin.

Economic adjustments of Cheddar cheese factories in Wisconsin are causing major changes in plant operations. The number of factories is decreasing and production per factory is increasing. This trend suggests the possibility of greater utilization of labor-saving techniques and devices.

Practical evidence indicates that the stirred-curd method of manufacturing Cheddar cheese may be more readily mechanized than the accepted matted-curd process of manufacture, but the characteristic openness of the stirred-curd type does not satisfy Wisconsin State Brand standards. Experimental attempts to correct this fault now are in progress. Tentative conclusions indicate that composition and flavor of normal stirred-curd Cheddar duplicate the conventional procedure. Texture is improved by maintaining temperatures approximating 100° F. during the entire draining process. Rapid cooling of the curd during this period promotes openness, inferior flavor, higher moisture, and slightly more acidity. Mechanical devices are being tried with some success to eliminate hand labor of the making process.

M26 The Use of Nonfat Dry Milk Solids in the Manufacture of Cheddar Cheese from High Fat Content Milk. G. H. WILSTER, C. E. JOHNSON, AND P. R. ELLIKER, Oregon State College.

Results on 54 batches of experimental cheese indicate that Cheddar cheese of high quality can be manufactured from high fat content pasteurized milk adjusted with reconstituted nonfat dry milk solids to a fat to solids-not-fat ratio of 1: 2.35. A high preheat type of powder was used. Preliminary studies also were carried out under commercial conditions. Some of the batches of cheese were made with dry milk manufactured by a low heat treatment method.

Body and texture defects, such as crumbly and brittle, occurred when milk containing more than 4.7 per cent fat was standardized with reconstituted nonfat dry milk solids to a ratio of fat to solids-not-fat of 1: 2.35. The addition of 0.025 per cent calcium chloride to the cheese milk, and holding the reconstituted nonfat dry milk solids for a period of time before addition to the milk were found to have no advantage. Excessive agitation during cooking was found to be conducive to a crumbly curd during cheddaring and to a low moisture cheese. After the whey was drained, the curd was allowed to mat for 15 to 20 minutes before the first turn was made. Further studies are being made to determine the type of nonfat dry milk that is most suitable for use in standardizing milk for Cheddar cheese.

M27 The Problem of Sampling Cheddar Cheese for Analysis. WILLIAM C. WINDER AND WALTER V. PRICE, University of Wisconsin.

Moisture in Cheddar cheese is not distributed uniformly and Cheddars from the same vat lot are not alike. The usual sample removed by trier from a Cheddar overestimates the moisture content of the edible portion of the cheese. In this study, commercial lots of Cheddar cheese were sampled with a long trier. Three plugs were taken from both flat surfaces of each Cheddar in the manner suggested in "Methods of Analysis", 6th Ed., AOAC. Each plug was analyzed; the average percentage of moisture in all plugs for a Cheddar was called the "moisture content" of that Cheddar.

The results show that, no matter how it is taken, the plug sample must be regarded as merely an estimate of the moisture content of the vat lot of cheese. The minimum sample for reasonable estimates consists of plugs which remove the vertical axis of the Cheddar, excluding only the rind portion necessary to seal the openings in the cheese. According to the data, such a sample, taken from one Cheddar of a normal vat lot, estimates the moisture content of the lot within control limits of 0.61 per cent. When two, three or five Cheddars of the vat lot are sampled in this same manner, the moisture content can be estimated within control limits of 0.44, 0.36 and 0.27 per cent, respectively.

- M28 The Influence of *Oospora lactis* in Promoting Changes in the constants of Cheese Fat during the Ripening of Cheddar Cheese. S. L. TUCKEY, W. O. NELSON, AND R. V. HUSSONG, University of Illinois.

Oospora lactis when grown with *Streptococcus lactis* in cream produces marked lipolysis of the fat. To determine its effect on the fat of Cheddar cheese during ripening, three lots of milk were made into six batches of cheese. One half of each lot of milk was made into cheese containing the *O. lactis*, while the other half served as a control batch. Three different concentrations of inoculum of *O. lactis* were used as follows: 0.05, 0.18 and 1 per cent. The inoculum was a 3-day-old culture of *O. lactis* and *S. lactis* in sterilized 35 per cent cream, which contained at time of use 4,900,000, 2,700,000, and 4,300,000 *O. lactis* per ml., respectively, for the three lots. The fresh cheese curd showed 4,000, 14,000, and 420,000 *O. lactis* per g., respectively.

The following fat constants were determined at monthly intervals: acid number (Breazeale and Bird), Reichert-Meissl, Polenske and saponification numbers. The fat of the three lots of inoculum showed marked rancidity as determined by taste and titration. However, in the lots of cheese containing the *O. lactis*, the acid number was the only fat constant showing any consistent change from the control lot which would indicate any difference in the hydrolysis of the fat between the lots. The batch containing 0.18 per cent inoculum had the least desirable flavor of the different lots of cheese. The inoculum at time of use had an "old cream" and "fermented" flavor. The "fermented" flavor persisted during four months of ripening. No rancid flavors were noted in any of the lots of cheese.

- M29 Studies of Sources of the Typical Flavor in Cheddar Cheese. HAROLD E. CALBERT AND WALTER V. PRICE, University of Wisconsin.

Twenty-eight lots of Cheddar cheese and eight lots of cheese of various other varieties were analyzed for diacetyl. A modified colorimetric method was used. Although diacetyl was found in all samples, 75 per cent contained less than 1 p.p.m.

The Cheddar cheese was divided into two groups depending on its flavor. Group I included all lots with no flavor defects: 78 per cent of this group contained less than 0.5 p.p.m. of diacetyl. Group II included lots with adverse flavor criticisms. The diacetyl content of the latter group varied from 0.2 to 3.35 p.p.m. A small quantity of diacetyl seems to be essential in the typical flavor and aroma of Cheddar cheese. These studies are being continued to determine the importance of diacetyl in typical cheese flavor.

M30 Influence of Temperature of Ripening on the Concentration of Tyramine in American Cheddar Cheese. A. C. DAHLBERG AND F. V. KOSIKOWSKY, Cornell University.

Two batches of pasteurized milk of excellent sanitary quality each were divided into three cheese vats and made into American Cheddar cheese by the usual procedure. Two per cent of Hansen's lactic starter was added to one vat of milk, 2 per cent of DK cheese starter (containing a special strain of *Streptococcus faecalis*) was added to another vat of milk, and 1 per cent of each of the starters was added to the third vat of milk. The cheese from each vat of milk was cured for 1 year at 40, 50 and 60° F.

The cheese was analyzed for chemical composition and bacterial content and was scored at regular intervals. In agreement with previous research from these laboratories, it was found that the least tyramine developed in the cheese containing the usual lactic starter, and the most developed in cheese made with both commercial lactic and DK cheese starters. Furthermore, the intensity of Cheddar flavor increased with increased tyramine content. The amount of tyramine in cheese increased as the temperature of curing increased and so did flavor development. Cheese made with commercial lactic starter cured for 1 year at 40° F. contained only 3 γ of tyramine per g. of cheese; at 60° F., it contained 37 γ per g. The highest tyramine contents were found in 1-year-old cheese made with both commercial lactic and DK cheese starters. This cheese cured at 50° F. contained 1,369 γ per g., and at 60° F. it contained 2,554 γ per g. cheese.

M31 Methods for Studying the Ripening of Cheese. H. H. SOMMER AND W. J. HARPER, University of Wisconsin.

The method of Van Slyke and Hart (1902) was modified by using a Waring-type blender in place of grinding with sand, to suspend the cheese in water, and by making the first separation by acidification to pH 4.7.

In the original Van Slyke and Hart procedure, the first separation was into water extract of the cheese at 50° C. and water insoluble residue. From the water extract was obtained a precipitate on acidification which they called "paramuclein". From the water insoluble residue a 5 per cent NaCl-soluble fraction which was called "unsaturated paracasein lactate" was obtained.

In view of the later findings of Van Slyke and Bosworth (1912) on the water and 5 per cent NaCl solubility characteristics of paracasein as influenced by reaction and calcium salts, the significance of Van Slyke and Hart's "unsaturated paracasein lactate" and "paramuclein" is doubtful. Various suggestions have been made that the water extraction of the cheese should be made with control of such factors as pH, NaCl concentration, and CaCl₂ concentration. However, since the fraction here involved is

essentially unhydrolyzed paracasein, it has little or no significance in studies of proteolysis in cheese ripening. Accordingly, in the present method, the first separation is the precipitation of materials that are insoluble at pH 4.7 (50° C.), and subsequent separations are applied to the filtrate using the general scheme of Van Slyke and Hart.

M32 The Effect of Added Amino Acids on Flavor Development in Cheddar Cheese. R. J. BAKER AND F. E. NELSON, Iowa State College.

The possibility that the addition of various amino acids to cheese curd made from pasteurized milk might furnish the substratum for production of the typical flavor and aroma compounds of a fine raw milk cheese was the basis of this study. Of the amino acids normally present in casein, alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophane, tyrosine, and valine were used. They were added with the last increment of salt, at the rate of 0.5 g. (0.04 per cent) to a 2.5-lb. quantity of curd, which then was pressed in special small hoops, giving a cheese 5 inches in diameter and about 2.5 inches high.

On the basis of organoleptic examinations every 4 weeks during a 24-week curing period for two complete series of cheese, histidine produced a definitely inferior cheese, having a pronounced unnatural flavor due to the amino acid itself. Glycine, methionine, tyrosine, serine, glutamic acid, arginine, aspartic acid and valine showed a possible tendency to increase the desirable flavor level in the test cheese. These eight amino acids were used in 0.5 g. (0.04 per cent) and 1.5 g. (0.13 per cent) amounts in a third series of cheese. Plate counts and direct microscopic counts also were made. No consistent and significant differences were established in this series.

Separate additions of 19 amino acids to the curd of Cheddar cheese made from pasteurized milk had no consistent and reproducible effects upon flavor development or bacterial growth.

M33 Studies of Amino Acids in Cheddar Cheese during Ripening. W. J. HARPER AND A. M. SWANSON, University of Wisconsin.

An investigation was made to determine some of the individual compounds formed by protein degradation during the ripening of Cheddar cheese. Analyses for nine amino acids were made by microbiological assay on hydrolyzates of cheese (I), water extract of cheese (II), and the water extract minus heat coagulable material (III). Determinations also were made for the "apparent free" amino acids in the unhydrolyzed water extract (IV). These amino acids were aspartic acid, glutamic acid, glycine, isoleucine, leucine, lysine, phenylalanine, proline and valine. Five repre-

sentative cheese, varying in age and degree of cheese flavor, were studied.

All of the amino acids were found to be present in every fraction (I, II, III, and IV) of each cheese. The quantity of all amino acids in II, III, and IV increased directly with age. Regardless of this increase, a direct relationship was found between the degree of cheese flavor and the majority of the different amino acids present in fractions III and IV. Further investigation is being undertaken to determine whether amino acids contribute to cheese flavor or are merely indicative of ripening changes.

M34 The Influence of Various Lactobacilli and Certain Streptococci on the Chemical Changes, Flavor Development and Quality of Cheddar Cheese. R. P. TITSLER, G. P. SANDERS, H. R. LOCHRY, AND O. S. SAGER, Bureau of Dairy Industry, U. S. Department of Agriculture.

Cheddar cheese was made from milk of good quality which was pasteurized and divided equally into two lots. Lactic starter was used in one lot and lactic starter plus a supplemental starter was used in the other lot, in 110 experiments. Bacterial (quantitative and qualitative), chemical (nitrogen fractions, titratable acidity, pH, lactose, biacetyl, moisture), and physical (plasticity, elasticity, tensile strength) determinations were made at regular intervals.

Streptococcus faecalis, *Leuconostoc citrovorum*, *Leuc. dextrans*, and *Leuc. mesenteroides* had little or no effect on proteolysis or quality of the cheese. *S. liquefaciens* greatly increased proteolysis and produced an objectionable flavor. *Lactobacillus casei*, *L. plantarum*, *L. brevis*, and *L. fermenti* grew rapidly in the cheese. *L. bulgaricus*, *L. helveticus*, *L. lactis*, and *L. acidophilus* were not detected after 2 weeks and had little or no effect on proteolysis or quality. *L. casei* increased the acidity and the rate of softening of the body but did not increase soluble nitrogen; it increased the development of flavor but later caused an acid flavor and "short" body. *L. fermenti* produced gas and a very objectionable flavor. *L. brevis* produced an objectionable flavor. Some strains of *L. plantarum* increased development of desirable flavor without producing excessive acidity and ultimately may prove useful for faster curing.

M35 Some Physiological Effects of Dietary Lactose.¹ JESSIE ELIZABETH FISCHER AND T. S. SUTTON, The Ohio State University.

The rate of passage of food residues through the digestive tract of rats was estimated by fecal recovery of a dye (Sudan III) which was administered by stomach tube. Food residues passed through the digestive tract more rapidly when lactose was administered by stomach tube than when sucrose was similarly given. A similar effect of lactose incorporated in

¹ Research supported by The American Dry Milk Institute, Inc.

the ration depended upon the percentage of lactose included. The order of rapidity of food residue passage produced by the different rations tried was: 30 per cent > 20 per cent lactose > 40 per cent lactose > basal corn-starch. The severity of diarrhea observed was greatest in the animals on the 40 per cent lactose ration despite the foregoing results. A 40 per cent lactose ration fed to young rats increased the amount of both water and dry matter in the contents of the digestive tract. Despite persistent watery diarrhea in the lactose-fed group, dehydration did not occur, although water intake was not increased.

Roentgenologic studies of the passage of a ration containing 60 per cent lactose indicated that the chief site of stasis was the cecum, although the stomach seemed to empty somewhat more slowly. Activity of the longitudinal muscle of isolated small intestinal segments failed to indicate that lactose exerts a direct stimulatory effect on this muscle.

M36 The Limitations of the Refractometer Readings of Milk Serums in Detecting Watered Milk. L. R. ARRINGTON AND E. L. FOUTS, University of Florida.

Refractometer readings were made of the serum of mixed Holstein and mixed Jersey milk and on the same samples after 3, 5, 7, and 9 per cent water had been added. Readings were made on the acetic serum, copper serum and sour serum as directed by the Association of Official Agricultural Chemists. Also, a modification of the sour serum method was made by adding 1 per cent culture and incubating the milk at 70° F. for 15 hours. The freezing points using the Hortvet cryoscope were determined on the original and diluted samples. The percentages of fat, total solids (Mojonnier) lactose and chlorides were determined on the original samples.

On the basis of standards listed in the official methods of the Association of Official Agricultural Chemists, added water in amounts up to 9 per cent in milk of normal fat and total solids content was not positively detected by any of the serum methods. The addition of smaller amounts of water to milk with low fat and total solids could be detected. The standards given for each of the official methods appear to be too high for normal milk. Sour serums prepared by the official method gave inconsistent values. When the method was modified by addition of 1 per cent culture followed by incubation for 15 hours at 70° F. the values were more consistent and averaged 1 refractometer degree lower than serums from natural souring.

M37 The Application of Flame Photometry to Determinations of Calcium, Potassium and Sodium in Milk. W. A. KRIENKE AND NATHAN GAMMON, JR., Florida Agricultural Experiment Station.

Two flame photometers were used in this study, one a combination of a Lundegardh burner with a Beckman quartz spectrophotometer and the

other a Perkin-Elmer model 52 A spectrophotometer. A comparison of results is given.

The procedure for analysis of milk for sodium and for potassium is to ash the sample, take up the ash in hydrochloric acid, evaporate to dryness, take up the ash in a known volume of 0.01 N hydrochloric acid, fiber, and atomize in the flame. The application of the internal standard technique involves the addition of a known quantity of lithium chloride before making to final volume.

Calcium presents a more difficult problem because of the depressing effect of phosphate and sulfate ions on the excitation in the flame. Two procedures, one a spectroscopic buffer, and the other a lead precipitation, are suggested for control of phosphate and sulfate ion variations. The importance of using standards containing the same ions and in approximately the same relative concentrations as present in the prepared samples is emphasized.

M38 A Rapid Method for the Determination of Nitrogen in Milk Products by Direct Nesslerization of the Digested Sample. J. H. HETRICK, Dean Milk Company, Rockford, Illinois, AND R. McL. WHITNEY, University of Illinois.

Interest in the changes in the protein fractions of milk made it necessary to develop a more rapid method for the determination of nitrogen than was currently in use. As developed, the test consists of: (a) digestion in a micro-Kjeldahl apparatus of a diluted sample with potassium sulfate and sulfuric acid until the sample clears, (b) completing the digestion with 30 per cent hydrogen peroxide, (c) diluting the digested sample using a gum acacia solution, (d) addition of a Nessler's reagent to an aliquot of this solution, (e) measuring the per cent transmission spectrophotometrically at 420 m μ , using a potassium dichromate solution as a standard blank, and (f) computing the nitrogen content from a standard concentration curve.

The effects of the following variables upon the determination were investigated: concentration of potassium sulfate, sulfuric acid, hydrogen peroxide, gum acacia, type and amount of Nessler's reagent, time or color development, and temperature of color development. Results of analyses using the technic indicate satisfactory reproducibility with a standard deviation of 1.5 per cent of the nitrogen content of the sample. When compared to the A.O.A.C. procedure, the results were found to average approximately 2 per cent below those of the official method. The saving in time, while dependent upon the apparatus available, is considerable.

M39 Studies on Separation and Fractionation of Casein. E. C. HAGBERG AND A. M. SWANSON, University of Wisconsin.

Studies have been made on the precipitation of casein and its frac-

tiation into *alpha* and *beta* casein by use of Warner's method. This method is based on the fact that at 2° C., *beta* casein has a higher isoelectric point than *alpha* casein. Fractional precipitation is accomplished by approaching the isoelectric point from the acid side.

The dried samples were prepared by lyophilizing. This resulted in material which was more readily soluble than that dried by alcohol and ether methods. Light transmission observations were made on the precipitation of casein and its fractions. The degree of dispersion as evidenced by turbidity varies with the pH, and the changes leading to acid precipitation were gradual. The relationship between turbidity and pH was different for the two fractions of casein. *Beta* casein was found to have a higher relative viscosity in the pH range of 5.5 to 9.0.

Analytical results showed that *alpha* casein contained a higher percentage of total phosphorus, aspartic acid, glutamic acid and tyrosine. Significant differences were found in rate of hydrolysis in alkali. Alpha casein was most readily hydrolyzed.

M40 The Fractionation of Milk Fat by Molecular Distillation. E. L. JACK AND MRS. L. B. OLSEN, University of California.

Milk fat has been subjected to molecular distillation using a cyclic still. The fat was passed over the distilling surface twice at each fractionating temperature. Attempts to distill exhaustively at each temperature were too slow to be practical. The first fraction was removed at 140° C. and the temperature was increased 10° C. for each succeeding fraction until substantially all the fat had been distilled. The last distillate was removed at 190° C., making seven fractions including the residue.

Analyses of the fractions obtained from a fat having a saponification number of 236 and an iodine number of 28.5 showed that the saponification number of the fractions decreased from 263 to 205 progressively with succeeding fractions, while the iodine number increased progressively from 16 to 43. The non-saponifiable components were segregated among the fractions. Fractionation by molecular distillation yields fractions of different chemical properties from those secured by solvent precipitation.

M41 The Measurement of Free Fatty Acids in Dairy Products. H. A. HOLLENDER, S. R. RAO, AND H. H. SOMMER, University of Wisconsin.

A method has been worked out for the estimation of the free fatty acids during lipolytic studies on fat in the form of emulsions. This method can be applied to the estimation of free fatty acids in powdered milk, cream and other high-fat dairy products. The proposed method utilizes the high solubility of free fatty acids in 95 per cent alcohol. Five to 10 g. of the

substrate (powdered milk or cream) is placed in a centrifuge tube of about 100 ml. capacity. Fifty milliliters of neutral 95 per cent ethyl alcohol are added, maintaining 85 per cent concentration of alcohol in the mixture. The mixture then is boiled for 30 seconds, cooled and centrifuged until clear. The supernatant is decanted off and the extraction repeated with fresh alcohol. The combined supernatants are titrated with 0.02 N alcoholic alkali, using a micro burette and 5 drops of 1 per cent phenolphthalein as the indicator.

Recovery studies on fatty acids added to milk and cream have resulted in quantitative recoveries of oleic, palmitic and butyric acids and mixtures of these. Further studies on the recovery of other fatty acids are being carried out, along with work to determine the amount of non-fatty acid acidity that will be extracted by the alcohol.

M42 The Determination of Linoleic Acid in Butterfat. P. S. SCHAFER AND GEORGE E. HOLM, Bureau of Dairy Industry, U. S. Department of Agriculture.

Samples of the fat acids of butter oil, from milks produced during the spring, summer, and winter months, were isomerized with alkali and their absorptions in the ultraviolet region of the spectrum, 224-274 m μ , determined. The linoleic acid content was calculated from the degrees of absorption at 234 and 268 m μ and the specific absorption coefficient of diene and triene conjugations at 234 m μ , and 234 and 268 m μ , respectively. In fat acid samples containing 1 and 3 per cent of added linoleic acid, the maximum deviations from theoretical recovery were 0.008 and 0.015 per cent, respectively. The linoleic acid content of the milk fats ranged from 2.11 to 2.40 per cent, the latter being the value obtained on milk fat produced during the summer months.

M43 Retention of Ascorbic Acid, Changes in Oxidation-reduction Potential, and the Prevention of an Oxidized Flavor during Freezing Preservation of Milk. R. W. BELL, Bureau of Dairy Industry, U. S. Department of Agriculture.

The tendency of milk to develop an oxidized flavor is a limiting factor in the preservation of milk by freezing. Oxidation-reduction-potential changes and decreases in ascorbic acid during the onset of the off-flavor in frozen milk were measured by examining the thawed product. A strong oxidized flavor developed in frozen milk with but slight decrease in ascorbic acid content, and the rate of development was much slower in milk that had been fortified with the acid. Deaeration aided in the preservation of ascorbic acid but only slightly retarded the onset of the off-flavor.

It was concluded that a low oxidation-reduction potential obtained by

adding ascorbic acid to fresh milk greatly retards but does not prevent the development of an oxidized flavor in frozen milk. However, it does not increase the retention of vitamin C in the form of ascorbic acid.

- M44 The Effects of the Treatment of Milk and the Subsequent Storage of Cream and Butter below Freezing Temperatures upon the Sensitivity of Fat to Oxidation as Determined by the Re-emulsification Test. VLADIMIR N. KRUKOVSKY, E. S. GUTHRIE, AND F. WHITING, Cornell University.

The milk fat (triglycerides) is relatively stable in fresh milk and is not involved in the reaction which produces the oxidized flavors. However, the fat undergoes oxidation in the presence of ascorbic acid, resulting in the development of the objectionable flavors and losses in vitamins E, A and carotene. The susceptibility of milk fat to this type of deterioration is determined primarily by the treatment of milk and cream, the type of product held (butter, cream, fat), and the conditions of storage.

Cream separated from milk depleted of its total vitamin C content by oxygenation during the pasteurization was found free of all flavors at the end of the twelfth month of storage at 0 to 3° F. However, the storage life of fat, as determined by the re-emulsification test, was terminated at the end of 4 to 6 months, depending upon the conditions of processing. Only fat from butter churned from cream pasteurized at 160 and at 170° F. and pure fat retained their abilities to resist this type of deterioration at the end of 2 years of storage at 0-3° F.

While the oxygenation of milk results in the prevention of oxidized flavors, the latter can be induced again by the addition of ascorbic acid.

- M45 Ascorbic Acid Oxidation in Milk by Preformed Hydrogen Peroxide
VLADIMIR N. KRUKOVSKY, Cornell University.

A study was made to ascertain if the inactivation of peroxidase in milk, as produced by heat (Zilva, at 76.7° C.), would result in non-reactivity of ascorbic acid and H_2O_2 and if the ability of milk to promote ascorbic acid oxidation by added H_2O_2 can be restored either by the addition of horse radish peroxidase, prepared according to Sumner and Gjessing, or of Cu.

While ascorbic acid was oxidized rapidly and completely by H_2O_2 added to milk pasteurized at 61.1° C. for 30 minutes, H_2O_2 was not utilized for the oxidation of ascorbic acid, neither in the milk pasteurized at 76.7° C. nor in milk to which H_2O_2 was added in excess of the amount needed to complete ascorbic acid oxidation prior to pasteurization of milk at 61.1° C. This fact was established by the readdition of both reagents to milk after the heat treatments. However, the foregoing reaction was induced again by the addition of peroxidase to non-reactive milk. Only a part of

ascorbic acid was oxidized rapidly by H_2O_2 with Cu as a catalyst in non-reactive milk.

These results indicate that peroxidase in milk may play an important part in the reaction involving ascorbic acid oxidation in the presence of added H_2O_2 .

M46 Stimulation of the Oxidized Flavor in Homogenized Milk by Daylight as Governed by the Vitamin C Content of the Milk. E. S. GUTHRIE AND VLADIMIR N. KRUKOVSKY, Cornell University.

A study was made of the susceptibility of homogenized milk to the development of the oxidized flavors when it was exposed to light in the presence or absence of vitamin C in milk. For this purpose some of the milk was first depleted of its total vitamin C content either photochemically or by hydrogen peroxide, and then pasteurized at 143° F. for 30 minutes.

Irrespective of the pressure, the quick partial photochemical oxidation of ascorbic acid resulted in the promotion of the oxidized flavors in the homogenized milk, whereas its complete oxidation resulted in the prevention of the oxidized flavors. The oxidized flavors were not induced by the addition of ascorbic acid to homogenized milk which was first depleted of its total vitamin C content by exposure to light and subsequent pasteurization prior to homogenization, providing the pressure used was above 1,000 lb. per square inch.

M47 A Study of Seepage from Bottles of Homogenized Milk. E. O. HERREID, J. FRANCIS, AND P. H. TRACY, Illinois Agricultural Experiment Station.

Milk sometimes appears in small amounts around the edge of the fiber disc cap and around the wire staple which anchors the paper tab on the bottle cap. Milk also sometimes appears on the lower edge of the metal cap which covers the mouth and lip of the bottle. In the market milk industry this condition has been referred to as seepage, leakage, creeping, crawling and swelling.

Fiber disc caps and bottle cap seats were measured and slight variations in their size and shape were observed, but even the most extreme variations could not be correlated with seepage. However, the fiber disc and metal caps varied in their ability to hold a tight seal, a factor which did affect seepage.

The principal cause of seepage is the expansion of gases and vapor in the headspace beneath the cap when the bottles are exposed to elevated temperatures. When seepage occurs, the cap usually is firmly set and the pressure reaches a certain point and is suddenly released, forcing out a small quantity of milk. Seepage usually does not occur when the pressure

is released slowly during exposure of the bottles to elevated temperatures. These changes in pressure were observed with a manometer attached to the bottles. The incidence of seepage is increased by the excessive incorporation of air in milk during processing operations and exposing it to elevated temperatures during hauling and delivery.

M48 The Leucocyte Count of the Complete Milking of Normal Animals for Complete Lactation Periods. E. O. ANDERSON, University of Connecticut.

The average arithmetic cell count of 19,710 samples from the complete milking of 18 mastitis-free cows over their complete lactations was found to be 160,000 per ml. as compared to a cell count of 380,000 and 220,000, respectively, for cows chronically infected with staphylococci and streptococci other than *Streptococcus agalactiae*. The average cell count of composite samples of the herd milk taken over the same period was 380,000 per ml.

The average arithmetic cell count of mixed herd milk obtained at the receiving deck of milk plants from 20 herds infected with *S. agalactiae* was found to be 880,000 per ml. as compared with a cell count of 660,000 per ml. in mixed milk from 11 herds free of *S. agalactiae*. From the data available, a parabolic curve fitted by the method of least squares indicates that when the percentage of *S. agalactiae* cows in a herd reaches 36 per cent or over the average cell count of mixed herd milk will probably be a million cells per ml. or more.

M49 Effect of Some Water Constituents on a Quaternary Salt. W. S. MUELLER AND D. B. SEELEY, University of Massachusetts.

This study was undertaken to obtain more information on the controversial problem of whether the constituents of water, especially those of hard water, affect the germicidal potency of quaternaries. Natural waters and also waters to which various common ions had been added were used to prepare a 200 p.p.m. quaternary solution, which was tested against *Escherichia coli*.

No close correlation was noted between water hardness as measured by standard soap titration and the germicidal potency of the quaternary. Differences in hydrogen-ion concentration found in the natural waters examined had no significant effect on the quaternary. The cations calcium, magnesium and ferric iron decreased the germicidal potency of the quaternary, while potassium, sodium and lithium had no adverse effect. Ferric iron was considerably more detrimental than calcium or magnesium, which have similar effects on the quaternary. When the water contained as much as 1000 p.p.m. of calcium or magnesium, the 200 p.p.m. of quater-

nary was sufficiently potent to give approximately 100 per cent kill on *E. coli* after 8 minutes contact, while as little as 10 p.p.m. of ferric iron completely inactivated the quaternary. The anions studied were chlorides, sulfates, nitrates and carbonates, and no adverse effect was noted.

While the study is being continued, the results to date indicate that when the quaternary is added to most potable waters, a concentration of 200 p.p.m. has sufficient reserve germicidal potency for most sanitizing jobs.

M50 Germicidal Effectiveness of Certain Hypochlorites and Quaternary Ammonium Compounds under Simulated Plant Conditions.
P. R. ELLIKER, Oregon State College, AND K. R. SPURGEON, Purdue University.

Germicides employed in this study were selected by three screening tests. The hypochlorites selected included one highly active and one slower acting compound. The quaternaries selected included one widely used commercial compound and a second pilot plant product of comparatively higher germicidal activity. Plant germicidal procedures were simulated by controlled trials with stainless steel dishes and experimental 50-gallon cheese vats. Exposure periods varied from 15 seconds to 3 minutes. Concentrations of germicide varied from 25 to 200 p.p.m.

Exposure for short periods comparable to germicidal treatment just before use of equipment indicated more rapid germicidal action by the hypochlorites than by the quaternaries. The difference was slight in certain trials with pure cultures of *Streptococcus lactis* and thermophilic micrococci, but was quite pronounced in numerous trials with coliform species. The fast acting hypochlorite exhibited more rapid germicidal action in every case than either quaternary compound. Results suggested the desirability of raising the temperature of the quaternary solution for rapid destruction of coliform bacteria on dairy equipment.

A number of trials were undertaken to study residual or bacteriostatic effect of germicides applied to equipment after regular cleaning and rinsing at the end of the day's operations. Results indicated that where the equipment dried rapidly, treatment with hypochlorites resulted in fewer surviving bacteria. However, where equipment remained in a moist state, the continued bacteriostatic and bactericidal action of the quaternaries resulted in lower numbers than when hypochlorites were employed.

M51 Sanitizing Milk Cans in Mechanical Can Washers. G. W. REINBOLD, S. L. TUCKEY, R. V. HUSSONG, AND P. H. TRACY, University of Illinois.

- M52 Some Factors Involved in Developing a Sediment Test for One-pint Samples of Cream Taken off the Bottom of the Original Container. BEN M. ZAKARIASEN AND RAY W. MYKLEBY, Land O'Lakes Creameries, Inc., Minneapolis, Minnesota.

New cream grading regulations in Minnesota have made it important that a rapid, accurate, and practical method be developed for making sediment tests on one-pint samples of cream taken off the bottom of the original container at the receiving room. A tentative method is presented, along with some of the data used in arriving at this procedure.

The tester used is the plunger type, having a one-quart capacity and graduated to hold one pint of diluting solution with one pint of cream. By using a diluting solution consisting of water at 150° F. for sweet cream and a 4 per cent sodium bicarbonate solution for sour cream, very satisfactory tests can be made. The method consists of drawing one pint of diluting solution into the tester, followed by one pint of cream, gently tilting tester to facilitate mixing of solution and cream, and forcing the solution through the lintine disc. The new tester and new heat-resistant lintine disc are described and shown. This method is being used in Minnesota as well as several other states and has been generally accepted in the routine operation.

- M53 A Skunk-like Odor of Bacterial Origin in Farm-separated Cream. T. J. CLAYDON, Kansas State College.

During quality studies on cream delivered by producers to buying stations in Kansas, a sample was obtained during the winter season that manifested a strong skunk-like odor. Such an odor has been recognized previously as a defect in commercial butter and has been attributed either to the development of *Pseudomonas mephitica* or to plants consumed by cows. The defect in question was found to be the result of associative action of two species of bacteria tentatively identified as a *Pseudomonas* species and *Streptococcus lactis*. These organisms, in combination, were capable of producing a skunk-like odor in milk, cream and butter. The development of the defect depended on the balance of organisms, growth temperatures and pH and also appeared to be affected by the extent of exposed surface of the product. Various objectionable odors, including rancid and putrid types, frequently developed.

Since the bacteria responsible for the skunk-like odor appear to be common in raw cream, the defect might develop in cream or butter when conditions contribute to a suitable balance of organisms. Both species of organisms readily are destroyed by heat and should not survive in properly pasteurized products.

M54 Coliform Bacteria in Butter. R. N. SINGH AND F. E. NELSON, Iowa State College.

The coliform counts on 294 samples of commercial butter were found to be related in only a very general way to the initial score or to the keeping quality of butter. Many butter samples had a coliform count of less than 2 per ml. Line run samples showed that salted butter with low coliform count frequently is obtained from cream which has been contaminated with considerable numbers of coliform bacteria. Coliform counts detected contamination early in the processing operations more accurately than did yeast and mold or total plate counts. Studies on three strains of *Escherichia coli* and two strains of *Aerobacter aerogenes* introduced in considerable numbers into cream before churning showed that strain of organism, amount of salt and temperature of storage all affected the coliform population of butter. The field of applicability of the coliform count for butter seems to be for use on line run samples to detect sources of contamination. Too many uncontrollable factors affect the coliform count of commercial butter samples to permit satisfactory use of the test for control purposes.

M55 The Effect of *Streptococcus lactis* and Coliform Organisms on Soluble Nitrogen in Milk. E. B. COLLINS AND F. E. NELSON, Iowa State College.

A study was made of the trichloroacetic acid-soluble nitrogen produced by four strains of *Streptococcus lactis*, one strain of *Escherichia coli*, and one strain of *Aerobacter aerogenes* growing separately and in combination. All cultures were isolated for the study, and only very active strains were used.

Inoculations of 0.1 per cent culture were made into skim milk which had been pasteurized for 20 minutes at 185° F. Cultures were grown at 30° C. in large-mouthed, screw-cap, 4-oz. bottles, and tests were made at 0, 1, 3, 7 and 15 days. Tests included soluble nitrogen, insoluble nitrogen, total nitrogen, titratable acidity, total plate count and coliform count.

Strains of *S. lactis* gave a small immediate rise in soluble nitrogen, which then continued to increase gradually throughout the 15-day test period until approximately 15 per cent of the total nitrogen was in the soluble form. The magnitude of the increase varied slightly with different strains of *S. lactis*.

E. coli and *A. aerogenes* caused decreases in soluble nitrogen during the first 1 or 2 days of growth. These deficits gradually were overcome, and with each organism more than 20 per cent of the total nitrogen was in the soluble form at 15 days. The maximum soluble nitrogen produced by *A. aerogenes* was greater than the maximum produced by *E. coli*.

The soluble nitrogen values resulting from the action of two strains of

S. lactis growing in combination with *E. coli* and *A. aerogenes* resemble an average of the values produced by the organisms growing separately, except that the values at 15 days were essentially the same as for *S. lactis* alone.

EXTENSION SECTION

E1 Report of Dairy Records Committee. C. R. GEARHART, Pennsylvania State College.

This four-part report will present the following points:

1. Status of Uniform Testing Outfit. J. F. Kendrick, Bureau of Dairying. The final report on the D.H.I.A. outfit was made last year, but there is additional information that should be mentioned.

2. Report on Results When Samples from One Milking are Used. C. T. Conklin, Sec'y., Ayrshire Breeders' Association. This is in the form of an investigation and will be a comparison of results from sampling one milking as compared with evening and morning samples.

3. Simplified Figuring in D.H.I.A. Work. J. D. Burke, Cornell University. This will deal not only with the results of short cuts or simplified figuring, but also with its effect on the supervisors and the attitude of the dairymen and breed associations.

4. Some Facts about Compensation Insurance, Health Insurance, Hospitalization, Social Security, and Income Tax Deductions for D.H.I.A. Supervisors. R. C. Jones, Bureau of Dairying. These facts will be presented in their legal aspects rather than how such practices are carried on in the individual states.

E2 Seven Years of Central Laboratory Testing. J. E. STALLARD, University of Wisconsin.

Seven years of production testing under the Central Laboratory Testing plan in Wisconsin have shown: (a) That more cows now are being tested than could be tested under the individual association plan and at less cost to both owner-sampler and standard type members. (b) That better work is being done in the laboratory than on the farm as to accuracy and volume. (c) That better book work seems to be the rule in Standard herds. (d) That testers can be paid larger salaries plus adequate travel allowance. (e) That testers prefer laboratory type to the old method. (f) That most old-type individual associations now are doing owner-sampler testing and quite a percentage of them have established individual laboratories in the homes of the testers.

Eight per cent of Wisconsin's producing cows now are being tested. Eighty-six per cent of the herds and 84 per cent of the cows on test are in cooperative central laboratory associations. Sixty-five per cent of the

herds and 59 per cent of the cows on test are on the owner-sampler plan. Fifty-nine of the 62 counties in Wisconsin having Dairy Herd Improvement Associations have central testing laboratories.

The major difficulties consist of finding enough testers or fieldmen, maintaining an adequate training program, and supervision in the field. Efficient systems, short cuts, and improved equipment are a result of this type of testing.

E4 Interdepartmental Cooperation on Dairy Extension. EVERT WALLENFELT, GEORGE WERNER, AND CARL NEITZKE, University of Wisconsin.

This paper describes the development, planning, and carrying out of an extension program through the cooperation of nine University departments involving thirty specialists. Other interested groups such as the Wisconsin State Department of Agriculture, American Dairy Association of Wisconsin, dairy manufacturing trade associations, Wisconsin Dairy Federation, Portland Cement Association, and electric power companies (both private and cooperative) participated.

Forty-eight county extension plans of work had milk quality improvement as one of their projects for major emphasis. Recognizing the need for more dramatic emphasis on the improvement of the quality of milk and dairy products, the Director of Extension appointed a University Dairy Quality Committee made up of members of nine University departments. This Committee was given the task of developing an integrated dairy quality program to fit the needs of the counties.

Among the first projects was the preparation of a series of exhibits for Farm and Home Week at Madison early in 1947. Many county agents who saw this exhibit asked that it be used at county fairs. The Committee felt that this exhibit was not well suited for fairs but that it might be developed as a traveling exposition to be shown during the winter months.

The success of this entire project is a striking example of what can be accomplished through cooperation of many groups in effectively planning and carrying out a far-reaching extension project. The interest stimulated by this exposition has set the stage for effective dairy quality follow-up work in the counties.

E5 New Methods of Selecting Calves for 4-H Dairy Club Work. RALPH PORTERFIELD, University of Maryland.

One of the main objectives in getting 4-H Dairy Club members started is that of locating good quality calves at reasonable prices. Several states have specific plans for selecting calves. In Maryland each breed association sponsors a calf selection event. It is a cooperative project involving

the breed associations and the extension service. Each breed association announces the date, place and time for holding the event. It also appoints a Calf Selection Committee and designates the price groups (*e.g.*, \$100, \$125, etc.). Price is based upon type, pedigree and age. The Extension Service makes a state survey among 4-H Club members to determine breed interest. This information is submitted to the respective Calf Selection Committee.

On the day of the event all calves are assembled at a central location and distributed by means of a drawing. Pedigree information is distributed to each club member. The club members inspect the calves in the forenoon and locate calves they prefer. In the afternoon they draw a number for position to select.

These events are referred to as "Ayrshire Calf Drawing", "Guernsey Calf Selection Day", "Holstein Heifer Party", and "Junior Jersey Jam-boree". A total of 120 purebred heifers have been distributed through these media for the 1948 Dairy Projects.

E8 Suggested Revision of D.H.I.A. Rules and Regulations. C. R. GEARHART, Pennsylvania State College.

The D.H.I.A. Committee has requested all states to review the Uniform Rules in the Supervisor's Manual (B.D.I.M.-Inf-26) and to express approval or disapproval. Suggestions for revision are requested. The committee will summarize the suggestions and, with the help of these suggestions, will present a set of rules for adoption or for further consideration.

THE FORTY-THIRD ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

R. B. STOLTZ, *Secretary-Treasurer*

The American Dairy Science Association assembled in Hardman Hall at the University of Georgia, Athens, Georgia, on Monday, June 14, at 9:30 A.M. H. B. Henderson, local chairman, introduced President Harmon W. Caldwell of the University of Georgia, who gave the welcoming address.

Association President Paul W. Tracy then was presented and gave the following address:

THE PRESIDENT'S MESSAGE

As President of the American Dairy Science Association, I wish to take this opportunity to extend to our hosts the deep appreciation of all the officers and members for granting us the opportunity to hold the forty-third annual meeting on the campus of the University of Georgia. Some of you have come hundreds of miles to participate in these meetings, and I am sure none of you will be disappointed either in the caliber of the programs or the wonderful hospitality these fine people will show us. You have all observed the way in which every one on this campus is working towards the one objective of making our stay in Dixie a pleasant one. It is a big undertaking to house, feed and entertain a family as large as ours has grown to be, and I want you to know, President Caldwell, that our Society greatly appreciates your willingness to take us in. We are very grateful to Dean Chapman and Professor Henderson for the splendid work they have done in planning for our visit here. The cooperation of your faculty and physical plant staff in caring for our comforts and entertainment during the week is also deeply appreciated.

It was with some concern that I accepted the honor bestowed upon me when I was elevated to the presidency of the American Dairy Science Association. However, I found I was unnecessarily apprehensive about my responsibilities. Our Secretary, or more correctly speaking, Business Manager, Professor R. B. Stoltz, is so efficient that there is little for the president to do but sign his name to a few official documents. This is a fine tribute to the excellent work that has been done by our secretary over the period of years that he has held this important post. His annual report is further evidence that he is an excellent business manager of our association. I assure you that the affairs of the society are in good hands as long as we have a secretary-treasurer as loyal to our cause and as capable in his duties as Bob Stoltz.

Probably the most important project of the Society is our JOURNAL. When we have a good editor we want to keep him. It was for this reason

that two years ago the official board was reluctant to accept the resignation of T. S. Sutton. Some of you probably do not realize how much time is required to edit a good magazine twelve times a year. The Society was fortunate in securing an able successor to Professor Sutton. We greatly appreciate the fine job being done by Gene Nelson and we sincerely hope that he will be able to take enough time away from his family and professional duties to keep up the good work for a number of years to come.

The success of our annual meeting depends largely upon the seriousness with which the sectional officers and committee members take their responsibilities. I wish them to know that I deeply appreciate the fine work they have done during the past year in preparation for this program.

The job of supervising the organization rests pretty largely upon the official board. Your officers cannot make any important decisions without their approval. They are the first ones to arrive at the convention and are in session much of the time they are here. They give personally of their time, and I am sure that we all appreciate their efforts.

We Owe Much to the Founders of Our Society.—The first meeting of those interested in teaching and research in the field of dairying was called by the late Professor W. J. Fraser at the University of Illinois on July 17, 1906, while the Graduate School of Agriculture was in session at that institution. Action was taken at this meeting to form the National Association of Dairy Instructors and Investigators (later called the American Dairy Science Association) with a membership of eighteen. It is interesting to recall at this time some of the conclusions reached at this meeting 42 years ago.

For instance:

“1. Beet sugar causes sweetened condensed milk to ferment.

“2. The precipitation of both cane and beet sugar in sweetened condensed milk can be prevented by avoiding abrupt changes in the heating and cooling of the product.

“3. Acid silage will cause the formation of acid salts in the casein of milk.

“4. If large amounts of silage are fed there will be an increase in the acetic acid content of milk.

“5. In some sections of Chicago you will find 20–40% of the milk watered or preserved with formaldehyde.”

Several needs were pointed out at this meeting such as:

“1. Knowledge of how to control moisture in butter and a quick method of determining moisture.

“2. More scientific knowledge on the subject of feeding cows.

“3. Better and safer market milk.

“4. Scientific knowledge of the principles involved in manufacturing condensed milk.

"5. Knowledge as to the food value of cheese."

One speaker pointed out that the text books on dairying contained many statements which lacked scientific proof as to their accuracy.

To these pioneers of our association we owe a great deal. They were naturally misinformed on some things just as we today no doubt have erroneous ideas regarding certain of our accepted scientific beliefs. However, they realized their shortcomings and were eager to learn the correct answers. It was this desire to learn and the resulting inspiration to younger men who had the privilege of being associated with the pioneers in scientific dairying, such as O. F. Hunziker, W. J. Fraser, C. H. Eckles, C. C. Hayden, E. S. Guthrie, C. E. Lee, and B. D. White, that have made possible the noteworthy advances in scientific dairying in this country. Let us never forget our obligations to those who founded our Society and laid the ground work for our present successes.

Scientists Should Help to Preserve Democracy.—There are a number of priceless heritages which we of the present generation have received from those who preceded us. Noteworthy among these is the faith that these men had in the future of dairying as an industry and the future of the American way of living.

Free enterprise, the profit system and unlimited resources have resulted in a national income in this country great enough to support a high standard of living for our population. This in turn has made possible the consumption of large quantities of milk, cream, butter, cheese, ice cream and other manufactured dairy products. Those of you who have traveled over the world either as civilians or members of the armed forces do not need to be reminded of the scarcity of dairy foods in most countries outside America. The future of dairying is very closely related to the future of democracy in the United States. There is no place for highly developed dairy enterprises in countries where all workers are regimented, where worker incomes scarcely supply the purchasing power needed to take care of the barest necessities of life, where the state owns or controls all land, where there is no free enterprise, and where the profit motive does not exist except possibly in the black market.

There is widespread unhappiness throughout the world. People lacking in economic security are easily influenced by disciples of political and social reforms who promise relief from the existing ills. It is unfortunate that the political leaders of the civilized countries of the world have made such limited progress in bringing about suitable working relationships between nations. How can permanent peace and security be secured for all peoples? No one, of course, has the answer, but surely there must be a way for intelligent people to work out the answers to these problems. Scholars have amassed a wealth of knowledge pertaining not only to the physical and

chemical properties of matter but also to the basic laws of human relations—political science, sociology and ethics. The physical scientists are being blamed by some people for the possible early destruction of civilization through modern warfare, but possibly it is only the scientifically trained men and women who have sufficient knowledge of the fundamental laws governing social relationships to lead this world to the economic security and spiritual and political freedom we all desire. This, it seems to me, is the most important challenge confronting all scientists today. We have a responsibility to society to be fulfilled, not only through our contributions to the specialized field of knowledge with which we happen to be associated but also by participation in efforts to secure good government and good living for all people. It is important that we continue to contribute to the stockpile of basic truths. But our efforts will be in vain if in our close attention to the assigned task we overlook the infiltration of the enemy whose aim is to sabotage the social and political freedom we have treasured for so many years in America. We should alert ourselves to these dangers and assume major roles in the fight to maintain freedom, not only in scientific research and teaching, but in our economic, social and political practices as well.

If the world ever needed leaders capable of sound thinking, it needs them now. We have in this country many organizations representing the various professional groups such as ours. Is there no way in which this tremendous force organized for the basic purpose of promoting the betterment of man through exact observation and correct thinking can be used to a greater extent by our political leaders in solving vital national and international problems? When a war crisis arises, the importance of scientists to the military is recognized immediately. Large numbers of scientists were drafted or invited into the inner sanctum at Washington to help win World War II. Possibly what is now needed is recognition by more of our political leaders that scientists might also be useful in helping to win the peace.

Type of Cooperative Action Important.—Much progress has been made in bringing about cooperative action between the various groups in the dairy industry. This is a commendable practice as long as the cooperative action is not directed towards the accomplishment of selfish motives. The group action of dairy farmers, processors and health officers in building trade barriers around their own community or state for the purpose of excluding the competition of those in the same industry located outside the chosen area does not promote a permanent type of prosperity in the industry. Nor does an organization whose purpose is to rob processors of a competitive type of dairy product, of their raw milk supply or of the market for their manufactured product promote the welfare of the dairy industry. Use of

political alliances to promote laws beneficial to dairying and dairy products, but detrimental to competitive industries, eventually makes enemies for the protected industry and gains friends for the competitor. All branches of the industry should work together for the common good of the entire industry with proper consideration of the welfare of the consumer. Laws proposed to regulate our industry should be carefully considered before passage. It should be kept in mind that the more selfish we are as an industry, the more difficulty we have in getting along among ourselves; and the more laws we have passed regulating our operations, the more rapidly we are approaching a public utility status in the dairy industry. If we are to maintain the good will and confidence of the consumer, our industry needs to pay less attention to the importance of proper alliance with pressure groups and work cooperatively to maintain our industry on a sound fundamental basis so that there can be no criticism of our objectives.

We need to work cooperatively to make possible the efficient production of higher quality milk, cream and dairy products. The chemurgy experts will need to be much smarter than they are today to take away the market for our products, provided we are in position to supply the consumer with cream, milk, butter, cheese and ice cream that is top grade and at a price he can afford to pay.

Dairy Farmer Entitled to Good Income.—The efforts of some organized dairy groups have been directed towards means of increasing the return to the dairy farmer for the milk and cream he produces. I do not question the honesty of the intent, but I do question the logic of methods directed entirely towards higher prices to the producer as the solution. There are a number of reasons why the farmer should be well paid for his labor. However, this reward should result from efficiency rather than from artificially fixed prices which bring about high consumer costs. We must not lose sight of the fact that it is the functions of both the producer and the distributor that contribute to the price the consumer must pay for milk, and both of these groups have a moral obligation to society to reduce this cost to a minimum. This obligation will become increasingly important as consumer purchasing power decreases and competition from milk product-substitutes becomes increasingly difficult to meet.

Milk and Cream Quality a Fundamental Problem.—Studies made at the University of Illinois and elsewhere have shown wide differences in the cost of producing butterfat on dairy farms. The founders of our society discussed in their first meeting the need for improving efficiency in dairy farming. Dairy Herd Improvement Associations which they pioneered have done much to encourage better breeding, feeding and management methods. Yet we still have too many "boarder cows." We still have too many two- and

three-cow cream producers. Extension forces of our colleges and the procurement managers of our dairy plants have much unfinished business ahead of them in the establishment of more efficient dairy farming. We need to bring about permanent cures where farmers are inefficient in the production of milk and where farmers are chronic violators of the sanitary principles of milk and cream production. These problems can be solved only by helping such farmers to appreciate the need for a complete reorganization of their master plan of farming. As Professor Fraser once emphasized, a farmer will be a good dairyman only when he has all his operations in balance. A few "pep talks" on high quality milk or cream production will be of little value unless the farmer knows and appreciates the importance of soil erosion, crop rotation, proper feeding and breeding methods, use of labor-saving devices, modern conveniences for his home as well as the milk house, application of sanitary methods in all phases of farm practices, and proper social and recreational programs for the members of his family. Dairy farming is not the easiest type of farming. While demand for milk is increasing, the amount produced in the United States is decreasing. This is a matter of great concern to processors and distributors of milk and its products. If we are to attract the right type of young people to the dairy profession and if we are to secure the milk needed to supply demand, we must encourage the general adoption of modern dairy farm practices and the establishment of more interesting living conditions for dairy farmers and their families. This will call for the cooperative effort of all branches of our university and industry extension services, not overlooking the role the rural sociologist should play in this program. An emotional approach to the problem is not sufficient. Young people today are realistic in their thinking.

Engineering to Play an Important Part in the Future Development of Our Industry.—In the evolution of the mammary glands of the dairy cow little consideration was given by nature to the fact that many thousands of years later man would have occasion to ship milk from one section of the country to another. As a result we are forced to heat, cool, package and transport millions of pounds of water each year in order to supply consumers with the milk solids they desire for nutritional purposes. We have attempted to correct this inefficiency by perfecting concentrated forms of milk, but with limited success. If all housewives would accept milk in the form of "evaporated," the cost of milk solids to the consumer would be materially reduced. However, until we can perfect a sterilized canned milk that does not have the objectionable cooked flavor, Mrs. Housewife will insist on being supplied with regular pasteurized milk. Theoretically, powdered whole milk should be the ideal product for the market. However, the consumer acceptance of this product will be in direct proportion to the success of our efforts to improve the flavor and physical consistency of the reconstituted

milk. The chemical or food engineer will play an important part in the development of new methods of processing milk and milk products which will improve quality and reduce marketing costs. It may be possible in the future to collect milk from farms only two or three times a week without damage to either the flavor or sanitary quality of the milk. We may be able to kill harmful bacteria and enzymes at the farm by some acceptable means other than heat. We may find it possible to condense milk at temperatures no higher than 60-70° F. and sterilize without any detrimental effect upon flavor. We may find it possible to perfect single-service containers that can be made from material even cheaper than wood pulp. By a highly developed system of mechanical devices it may be possible to dump, sample and weigh milk automatically. It might then flow automatically and continuously through the plant guided by a master control panel board. Cleaning and sterilizing may be done in the same manner. Similar mechanical perfections in the processing and packaging of butter, cheese and ice cream offer a fertile field for experimentation. Already some progress is being made along the lines suggested. The next few years may bring forth some startling innovations in the field of dairy engineering.

Problems in Teaching and Research.—The marvelous developments made in the physical, chemical and medical sciences during the past 10 years serve as a challenge to our industry. The huge sums of money made available by the government for research in these fields not only have attracted top-notch scientists but have made available the facilities of extremely well-equipped laboratories. Research workers in these basic fields are setting a fast pace for those of us working in the applied fields. To hold our own we will need more and better equipped laboratories and expertly trained men and women for these laboratories. There is a definite shortage of well-trained dairy research workers in the industry at this time. There is an inadequately supply of young men entering this field. To get better-trained research workers we will need to do one of two things—train our own or bring in students from the arts and science colleges. If we train our own, we will probably need to offer two types of curricula, one for the students not scientifically minded but who wish to return to the farm or prepare for work in industry, the other which will give the student very little training in the practices of dairying but will supply essentially a basic training in arts and sciences. Matters will not be so simple as this, however, as it may be difficult to get students to register in colleges of agriculture to take a basic science course. Most of the better students interested in basic science will very likely prefer to register in colleges where they can major in mathematics, chemistry, physics or medicine. Industry or government subsidy of promising students who can be induced to go into the applied scientific fields may help to solve the problem. At any rate, something must be done to better

acquaint good students with opportunities in the dairy field for those trained in fundamentals of the basic sciences.

Reorganization of our agricultural colleges may be another approach to this problem. For years departments, in some colleges at least, have been organized on the basis of the products of the farm, that is, dairy husbandry, animal husbandry, agronomy, horticulture and the like. Considering the extent to which basic research is moving in to supplement and to some extent replace applied research, possibly we should change our organization pattern to a functional type. In this type of organization we could have such departments as chemistry, bacteriology, engineering, economics, physiology, soils and nutrition, with the research and teaching emphasis directed towards the problems of agricultural pursuits. Most of the farm practice courses now taught in colleges could be delegated to the secondary schools and high schools.

There is great need for better laboratory facilities in universities carrying on teaching and research in the field of dairying. The day is past when research can be done with facilities limited to a Babcock tester, a herd of cows representing the common breeds of the state, a bomb calorimeter, a balance or two, a few microscopes, some petri dishes, a Kjeldahl apparatus, a churn, a freezer, a cheese vat, a pasteurizer and cooler, a milk bottler and a \$10,000 creamery balance to turn over to the University authorities at the end of the fiscal year. If dairy scientists are to do classical research, they will need as good tools to work with as will those engaged in basic research in the fields of chemistry, physics and medicine. If this is not done, basic research will be moved out of our laboratories into those better equipped.

The main agricultural problems confronting the world today are: (1) the increasing population, (2) the decreasing food supplies, (3) decreasing soil fertility and (4) need for supplying people with food that will maintain optimum conditions of health and vigor. These problems have social, economic and political ramifications and are extremely important to the future welfare and happiness of all people. The satisfactory solution of these problems will call for group action on the part of our best physical and social scientists. Dairy products make up 20 to 25 per cent of the food intake of this country. It is extremely important, therefore, that we organize our research program in such a way that we can contribute our share of the answers.

I wish to mention certain specific problems that may have some influence in shaping the future plans for research and teaching in the field of dairying. Scientific developments which might be used in the destruction of armies and urban centers have been so publicized that the mere mention of war gives us a creepy feeling. Yet, consideration may need to be given to such problems as the possible effects of radiant energy and biological warfare not only upon the body functions of the dairy cow but upon the quality of

the milk she secretes. The possibilities of this type of warfare may lead to the re-location of important food factories, such as large milk bottling plants and evaporated milk factories. Possibly decentralization of such food enterprises would add to the national security.

How will present trends in our social and political pattern affect our teaching and research programs? Will organization of farm labor raise production costs so that the price of milk will be pushed beyond the reach of many consumers? Will the complete organization of dairy plant workers prevent the college-trained men from entering the industry through the usual channel? Will government control of the buying and selling of milk work to the advantage or disadvantage of the industry as a whole? Should there be one quality standard for all milk regardless of its use? Would a return to whole milk creameries help restore butter to the popularity level it once enjoyed in this country? Will the future development of our industry depend upon our ability to devise means of producing and marketing milk and its products more cheaply or upon the results of basic research in the fields of biochemistry and human nutrition which may reveal facts regarding the food value of milk not before understood?

Will the centralizing of industry in the hands of a few large companies lead to more government control? How will the growing trend of big industries to promote their own research programs and train their own personnel affect the objectives of the college department, particularly in the field of dairy manufactures? What type of research should be done by industry? What should be our objectives in training college students? How can the field of dairying be made more attractive to capable young people deciding upon their life work? These are a few of the important problems that our leaders of today need to consider.

The need for food has been the driving force behind all races of men. It has been the controlling factor in the survival of all species of wild animals. It is the main cause of most of the unrest in the world. We have plenty of food in this country today. However, in order to protect our own standards of living, it is imperative that we take immediate steps to help the less fortunate people of the world secure for themselves proper food and living conditions. Greater food production, not only in America but the world over, is a must; otherwise continued struggle of one class or nation against those in other groups or countries will continue to sap our resources. Dairying is one of the most important of food industries. We must ever continue to exert our energies to bring about more efficient use of our lands in the production of milk of highest nutritional value, and we must continue our efforts to improve the methods followed in giving milk and milk products the necessary form and place utility. Progress in the science of dairy farming and dairy manufacturing must keep pace with progress in other scientific fields if our production is to survive in the social and economic order that lies ahead.

After a musical number by Prof. Robert I. Harrison of the Music Department, University of Georgia, the Dean of the College of Agriculture, Paul W. Chapman, gave a most interesting talk.

There were 402 members present.

GENERAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

Athens, Georgia, June 16, 1948

President Tracy called the meeting to order at 3 p.m. in Hardman Hall. There were 218 present.

REPORT OF THE EXTENSION SECTION

At the opening session of the Extension Section held June 14, chairman E. H. Loveland appointed for the Resolutions Committee, Heebink, Arnold and Reaves and for the Nominating Committee, Brownell, Copeland and Linn. A symposium was held on testing and dairy records with reports on standard testing equipment, simplification of records and responsibility of dairy herd improvement associations for various taxes and insurance.

At the second session an illustrated talk on interdepartmental cooperation on dairy extension was presented, followed by the presentation of Extension Exhibits on Teaching Methods. Twelve states presented exhibits in this feature of the program. The report of the Teaching Methods Committee, presented by I. L. Parkin, committee chairman, was adopted.

At the joint session of the Production and Extension Sections held Tuesday afternoon, a symposium on reproductive problems in dairy cattle was held. A report on the activities of the Reproduction Committee of the Dairy Cattle Breeding Research Council of the Purebred Dairy Cattle Association was given by P. H. Phillips, and a brief talk on the program of the Purebred Dairy Cattle Association was made by G. A. Bowling, secretary.

The report of the joint committee on Dairy Cattle Breeding concerned recommendations for methods of sire proving and evaluation and a report on the new rules governing artificial breeding of purebreds. It also recommended the establishment of a joint Production and Extension Committee on type classification to give attention to problems in type as a factor in herd and breed improvement. The report was accepted as read. Enos Perry, chairman of the Dairy Cattle Breeding Committee, will send a copy of the complete report of this committee to each state within a short time.

Reports of the Breeds Relations Committee and of the Dairy Cattle Health Committee were read and adopted. (See report of the secretary of the Production Section.)

On Wednesday morning, 4-H club work was taken up as to national and regional contests, systems used in obtaining 4-H calves, and adoption of

practices as a result of 4-H dairy work. Joe Nageotte, committee chairman, acted as leader. In discussion of a national 4-H dairy judging contest, Heebink moved that the 4-H Committee for 1949 contact the 4-H Committee of the National Land Grant College Association relative to approval of a national 4-H contest. The motion was passed.

Charles Gearhart, chairman of the Dairy Records Committee, presented proposed changes for DHIA testing rules, which were taken up and discussed. A motion that the changes as revised in the meeting be adopted was made by J. D. Burke. The motion was passed.

At the business meeting, the report of the Resolutions Committee was read and adopted. Following the report of the Nominating Committee, Raymond Albrectsen of New York was elected secretary for the coming year; other offices are to be filled by succession from secretary to vice-chairman and vice-chairman to chairman.

Gerald Heebink, incoming chairman, named the new members on the various committees and recommended that the 1948-49 committees hold a meeting before leaving Athens.

The recorded attendance was 79, with 32 states and provinces represented.

Respectfully submitted—E. H. LOVELAND, *Chairman*; GERALD HEEBINK, *Vice-chairman*; C. W. REAVES, *Secretary*

Upon motion duly seconded, the report was accepted.

REPORT OF THE PRODUCTION SECTION

The Production Section held eight sessions at which some 75 papers were presented. It was necessary to have two sessions running concurrently because of the large number of papers.

In addition, a symposium on "Reproductive Problems in Dairy Cattle" was held at a joint session of the Production and Extension Sections. Papers on "Infectious Diseases as a Cause of Infertility", by D. E. Bartlett, "Functional Causes of Infertility and Methods of Treatment", which included discussions of hormonal and nutritional aspects by S. A. Asdell and the effects of inheritance by L. O. Gilmore, and "Possible Modes of Approach to the Study of Infertility", by J. F. Sykes, were presented at this session. In addition, P. H. Phillips reported on the activities of the Reproduction Committee of the Dairy Cattle Breeding Research Council of the Purebred Dairy Cattle Association. G. A. Bowling presented the program of the Purebred Dairy Cattle Association.

The following reports were presented and accepted by the Production and Extension Sections while in joint session.

The Report of the Breeds Relations Committee covered the activities of the committee in conjunction with the Purebred Dairy Cattle Association as related to "Uniform Rules for Official Testing". These rules have been revised and reprinted.

The Report of the Dairy Cattle Breeding Committee was read and accepted.

The symposium was sponsored by the Dairy Cattle Health Committee. The lack of fundamental information and the need for sound experimental studies were stressed in order to provide dairymen and research workers with adequate information on the prevention and correction of reproductive disorders. The need for continued research and educational activities in relation to the control and eradication of brucellosis, mastitis, calf losses and other diseases was indicated. Cooperation by the various interests is needed if rapid strides are to be made in the promotion of herd health.

The Committee on Resolutions of the Production Section consisted of Dwight Espe, R. E. Horwood and C. E. Wyle, chairman. The resolutions presented by this committee were read and approved.

The Nominating Committee of the Production Section consisted of D. M. Seath, I. R. Jones and K. L. Turk, chairman. Nominations for secretary were presented. L. O. Gilmore was elected. Vice-chairman L. A. Moore succeeds as chairman and secretary G. M. Cairns succeeds as vice-chairman.

The Report of the Dairy Cattle Judging Committee was presented by R. E. Johnson. He moved that the Production Section recommend to the American Dairy Science Association that the Dairy Cattle Congress at Waterloo, Iowa, be designated as the National Intercollegiate Dairy Cattle Judging Contest. This would be subject to the approval of the management of the show. The motion was duly seconded by D. M. Seath and approved.

It was voted to reactivate the Forage Committee to work with the related societies on problems in this field.

It also was voted to appoint three men to the Type Classification Committee that would work in conjunction with three men appointed from the Extension Section.

A motion made by K. L. Turk and seconded by C. Y. Cannon that the Program Committee of the Production Section abide by the rule adopted by the section several years ago to the effect that no author's name will appear on more than two papers to be presented at the section meeting was passed.

Respectfully submitted—G. H. WISE, *Chairman*; L. A. Moore, *Vice-chairman*; G. M. CAIRNS, *Secretary*

Upon motion duly seconded, the report was accepted.

REPORT OF THE MANUFACTURING SECTION

The programs of the Manufacturing Section were held in accordance with program published in the May issue of the JOURNAL OF DAIRY SCIENCE. Of the 55 papers scheduled, only two papers (M23 and 51) were not presented. A symposium, headed by K. G. Weckel, on certain phases of sanitation in the Dairy Industry also was held, with special papers being pre-

sented by representatives of industry, government and university research groups.

The business meetings of the section were held in accordance with the schedule, with Chairman P. R. Elliker presiding. Standing committee reports were received from the following standing committees: (a) Committee on Milk and five sub-committees; (b) Committee on Butter; (c) Committee on Products Judging; (d) Committee for Standardization of Tests for Dairy Alkalies and Methods of Reporting Results.

In addition to the adoption of reports, the following motions were made and passed:

1. That all standing committees be continued for the coming year.
2. That committee chairmen be encouraged to prepare their reports in suitable form for publication in the JOURNAL OF DAIRY SCIENCE.

At the final business meeting the following officers were elected: J. H. Hetrick, secretary; D. V. Josephson, the secretary of the past year, succeeds to the vice-chairmanship, and the vice-chairman for the past year, E. M. Barker, succeeds to the chairmanship for the coming year.

Respectfully submitted—P. R. ELLIKER, *Chairman*; E. M. BARKER, *Vice-chairman*; D. V. JOSEPHSON, *Secretary*

Upon motion duly seconded, the report was accepted.

EDITOR'S REPORT

The twelve issues of volume XXX of the JOURNAL OF DAIRY SCIENCE printed during 1947 consisted of 830 pages of original articles, 13 pages of Association announcements, 15 pages of program for the annual meetings, 31 pages of proceedings of the annual meetings, 81 pages of abstracts of papers presented at the annual meetings, 39 pages of indices, 50 pages of membership list, 192 pages of abstracts and 7 pages of miscellaneous. This makes a total of 1,258 pages, exclusive of the advertising sections and blank pages.

The material printed included 93 manuscripts (58 in the production field and 35 in the manufacturing field), 103 abstracts of papers presented at the Annual Meeting and 475 abstracts of literature appearing in the Abstract Section. Of the 125 papers submitted for publication during the year, 12 were rejected and 45 were on hand at the end of the year in various stages of processing for publication. The volume of material in all categories was greater than during the preceding year, indicating that a return to prewar volume of published material is only a matter of a relatively short time.

The editor wishes to express his appreciation of the assistance which members have given, in many cases anonymously, in the handling of the affairs of publication. Particularly to be commended is the thoroughness with which the majority of the reviewers have carried out their work in

helping to improve the quality of the manuscripts and the cooperative spirit in which authors have accepted the suggestions of the reviewers. Mrs. Phyllis McKimpson has been of great assistance to the editor, both editorially and in the handling of a multitude of details.

Arrangements for the reorganization of the Abstract Section had been virtually completed, to be effective on January 1, 1948, when negotiations with British DAIRY SCIENCE ABSTRACTS for some form of cooperation were reopened at their suggestion. In view of the uncertainties thus introduced, the editor deemed it inadvisable to make any changes in organization or to attempt to expand coverage until such time as the future policy of the Abstract Section was decided by the Journal Management Committee and the Board of Directors. A clear-cut decision for either expansion or termination is essential. Correspondence concerning relationships with the Milk Industry Foundation and the International Association of Ice Cream Manufacturers led to a decision to ask members of these organizations to meet with the Journal Management Committee for clarification of future policies.

The slowness with which some issues of our publication have appeared is regretted. The editorial office has gotten material to the printers on schedule and has returned all materials to the printers within the shortest possible time, frequently well under the time ordinarily scheduled for editorial operations. The hope is expressed that publication will resume normal schedule soon.

Four issues of the News Letter were sent to all members and student affiliates during the past year. While numerous favorable remarks have been made to the editor about this publication, the lack of response in the form of printable news items from the great majority of our members leads the editor to recommend the dropping of this project unless the members of the Association express a definite desire to the contrary and the heads of departments and others in similar positions are willing to assume the responsibility of sending in appropriate news items regularly.

Respectfully submitted—F. E. NELSON, *Editor*

Upon motion duly seconded, the report was approved.

SECRETARY-TREASURER'S REPORT

Membership and Circulation. The report of our membership and total circulation for the past year and the first half of 1948 excels any previous 18-month period, the total being 3,675 for 1947. Thus far in 1948 we have a circulation of 3,877, which is 200 more than the total for 1947. We are not bringing our association to the attention of all the dairymen in this country. There are at least 5,000 dairy scientists and commercial dairymen who would be members of the Association or subscribers to the JOURNAL if they were familiar with this opportunity.

During the past 2 years the cost of publishing the JOURNAL has increased almost 50 per cent, and the management does not believe it advisable to raise dues, subscriptions or advertising rates. We feel quite sure that if the members as a whole are interested in bringing the opportunity of membership and subscriptions to other dairymen who are seeking information, we can increase our membreship sufficiently to meet the increased cost.

The cost in 1948 will exceed our intake, especially when we insert the cost of publishing our 10-year index, but in the past we have accumulated earnings for the express purpose of taking care of our JOURNAL when the pinch came. In one or more years from now, unless printing costs decrease or unless our circulation increases, we will be forced to increase our membership and subscription rates.

The following data are a summary of our gain and loss in members for the year 1947:

Membership, December 31, 1946	1583
Gain: New members, 1947	139
Former student affiliates	46
Total gain	185
Loss: Members resigned and delinquent, 1947:	
Delinquent	83
Resigned	16
Deceased	6
Total loss	105
Net membership gain	80
Membership total, December 31, 1947	1663

It is interesting to note where the new members came from last year. Eighteen states gained 84 per cent of all the new membership. The following is a list of the new members by states for the year 1947:

New York	22	California	6	Washington	4
Illinois	17	Minnesota	6	New Jersey	4
Wisconsin	17	Missouri	6	Texas	4
Ohio	12	Michigan	6	Dist. of Columbia	4
Pennsylvania	10	Massachusetts	5	Florida	3
Canada	8	Tennessee	5	Montana	3
Maryland	7	Arizona	5		

The stimulus that our meeting in Georgia has had has already indicated its effect on the membership for the first 5 months of 1948. We have had 140 new members in that length of time and South Carolina leads all states by submitting 19 new members. B. E. Goodale and J. P. LaMaster are to be congratulated on the splendid team work. Their names are signed on all 19 membership blanks. They solicited these new members. I am not

familiar with the letter of solicitation, but I am sure that they will be glad to send you a copy. The people in South Carolina are not any more dairy-science minded than they are in other parts of the states, but in South Carolina they have two men who have interested more dairymen in the Association.

H. D. Lindquist of Massachusetts also is to be congratulated for the ten new members from that state. Georgia also added ten new members. California and Florida tied for fourth with eight new members each. The following states have three or more new members each:

Ohio	Wisconsin	Iowa
Michigan	Indiana	Maryland
Kentucky	Texas	Louisiana
Rhode Island	Oregon	Alabama

One more individual I would like to mention is M. H. Campbell of Rhode Island. He submitted four new memberships this year, thus doubling the membership in Rhode Island. Before Campbell went to Rhode Island, they had only two members.

In the first 5 months of the year, 17 states have submitted 100 new members out of a total of 140, or 71 per cent. We trust that these 17 states will continue their membership drive, and we also trust that the other 32 states will make an effort to increase their membership and number of subscribers in their respective states. This is the first year the Association has been able to take new members and supply them with back Journals after July 1. We are expecting to receive an additional 200 members and subscribers before the end of the year.

Student Branches. At the present time we have 16 student branches of the American Dairy Science Association at the following schools:

University of California	University of Missouri
University of Connecticut	Washington State College
Cornell University	Ohio State University
University of Florida	Oregon State College
University of Georgia	Pennsylvania State College
Iowa State College	University of Tennessee
University of Kentucky	Texas Tech College
University of Massachusetts	Virginia Polytechnic Institute

This year the Executive Committee granted certificates to the following schools: Alabama Polytechnic Institute, Michigan State College, and Montana State College.

Back Copies. Last year the income from back copies decreased from \$2,033 to \$1,880. Thus far in 1948 the figures show that our back copy sales will continue to decrease. The Association has replenished its stock of some of the early journals during the past year by purchasing them from members. This amounted to \$137.50. Our present inventory shows that

we do not have complete volumes of nos. 11, 29 and 30. By reproducing six numbers of back copies we would be able to furnish all back numbers. We recommend that they should not be reproduced until printing costs decrease. Our inventory shows the Association has on hand 31,392 individual journals in addition to the 20-year index.

Advertising. The total income from advertising in 1947 did not increase over 1946; however, we had 198 pages in 1947 versus 188 in 1946. In 1938 our present rates were set, at which time we had a circulation of 2,200 journals per month. Now we are printing 4,400 journals, or just twice as many, and are charging the same rate per page. We recommend that we increase our advertising pages by about 25 per cent this year and next so that we may bring our income from advertising up to about \$10,000 per year. We are pleased to take this opportunity to again express our grateful thanks to the organizations that use our journal as an advertising medium. Any courtesies shown our advertisers will be greatly appreciated.

Financial. The financial picture for 1948 is not encouraging. Our estimated expenses, due largely to increased printing costs and increased papers published, will amount to about \$35,500. Our estimated income is only about \$30,000. We are facing this year's financial situation with the thought of losing money and drawing on our reserve. In 1947 we had an income of \$28,221.06. This was an increase of more than \$2,000 in excess of the previous year. Our expenses for 1947 also excelled any previous year by almost \$5,000. They amounted to \$26,029.36, leaving an operating profit of \$2,191.70 on December 31, 1947. Our net worth on December 31, 1947, was \$38,085.21. Our investment in government bonds was \$36,287.00. A complete report of the Certified Public Accountant was sent to each member of the Executive Committee on March 10, 1948.

The Secretary wishes to take this opportunity to express his gratitude and thanks to the officers and members of the organization for the splendid cooperation that they have continued to give him.

Respectfully submitted—R. B. STOLTZ, *Secretary-Treasurer*

Upon motion duly seconded, the report was approved.

AUDITING COMMITTEE REPORT

The president then requested the report of the Auditing Committee, which was given by D. V. Josephson.

April 23, 1948

To the Directors and Members of the
American Dairy Science Association

Gentlemen:

On April 19, 1948, the Auditing Committee of the American Dairy Science Association met with Mr. Walter C. Burnham, a certified public

accountant who had examined the financial statement submitted by our secretary-treasurer.

Mr. Burnham reported after a thorough examination of the records, bank statement and checking account that all were accurate and in good order.

The Auditing Committee is satisfied that the financial statement for the year 1947 is correct and recommends that it be accepted by the Board of Directors and the members of the American Dairy Science Association.

Respectfully submitted—R. A. LARSON, W. J. BRAKEL, D. V. JOSEPHSON

Upon motion duly seconded, the report of the Auditing Committee was accepted and ordered filed.

REPORT OF THE JOURNAL MANAGEMENT COMMITTEE

In order to make the JOURNAL OF DAIRY SCIENCE better serve the membership and still come within the cost permissible without increasing subscription rates, the following recommendations are made:

1. That the Abstract Section be expanded to more thoroughly cover the dairy literature.
2. In order to economize on the cost, that: The Abstract Section and membership list be published in double column and reduced type size.
3. Due to the apparent lack of interest in furnishing the editor with news, that the News Letter be dropped.
4. That the editor be empowered to subscribe to, or negotiate exchange of, the JOURNAL OF DAIRY SCIENCE for foreign dairy journals, the value of which is not to exceed \$75.00 annually.
5. That the Journal Management Committee select associate editors on a staggered 4-year term basis—two to be chosen each year and no one eligible for appointment for two consecutive terms.

Respectfully submitted—P. R. ELLIKER; T. S. SUTTON; R. B. STOLTZ, *Ex-officio*; F. E. NELSON, *Ex-officio*; W. E. PETERSEN, *Chairman*

Upon motion duly seconded, the report was approved.

REPORT OF NATIONAL RESEARCH COUNCIL

During the past year, Robert F. Griggs, who served so ably as chairman of the Division of Biology and Agriculture, retired from that position. J. S. Nicholas, Sterling Professor of Biology, Yale University, is the new chairman.

In his opening address before the recent annual meeting of the Division of Biology and Agriculture, Dr. Nicholas urged that the member societies become more militant in pushing activities in their fields. He cited the situation with regard to the two and one-half million dollars which has been assigned by the Atomic Energy Commission for fellowships in five different areas, two of which deal with Biology and Agriculture. There were only 19 applicants for fellowships in biology and agriculture while there were 91

applications for training in the physical sciences. He suggested that this imbalance in applications for fellowships may be related to the higher salaries now being paid the physical scientists when compared to the biologist.

Dr. Nicholas also pointed out that the Veterans' Administration has requested the Division to investigate the agricultural training now being given under the G. I. Bill and make recommendations to them about it.

The announced policy of the Division is to put more emphasis on short-term projects which will give quick returns; although long-time projects seeking basic facts have great value, they do not attract funds as readily as do the short-term projects.

The Division through its Agricultural Board attempts to make essential contributions towards the solution of vital problems of agriculture. This is done through the activities of selected committees. The problems relating to dairying that are being studied at present are the "Public Health Aspect of Brucellosis", "Losses among Calves" and the "Dangers of Importing the Virus of Foot and Mouth Disease in Meat and Other Animal Products".

Already many of the members of the Dairy Science Association are acquainted with the objectives and organization of the American Institute of Biology, which was formally established on February 20, 1948, with twelve societies accepting full membership and one society adhering as an affiliate member. The National Research Council sponsored this organization of the Institute with the hope that it would function in the public relations field, where the Division as it now is organized cannot enter. Naturally, the hope is that all societies which now are affiliated with the Division of Biology and Agriculture in the National Research Council eventually will join the Institute of Biological Sciences to give it strength to fulfill its objectives.

Respectfully submitted—C. Y. CANNON

Upon motion duly seconded, the report was approved.

NECROLOGY COMMITTEE

K. M. Renner, Professor and Head of the Department of Dairy Manufactures, Texas Technological College, Lubbock, Texas, died September 2, 1947, following a cerebral hemorrhage. He received his B.S. degree from Iowa State College in 1921, and after serving on the Dairy Department staff at Kansas State College until 1927, he received his M.S. degree at Kansas and joined the staff at Texas Technological College, heading the Dairy Manufactures Department there until his death. He was active in civic, social, scientific and religious organizations. He is survived by Mrs. Renner, one son, two daughters and one brother.

Alan Leighton was born in Concord, New Hampshire, March 26, 1890, and died at Cottage City, Maryland, on February 11, 1948. He graduated from the University of New Hampshire and took graduate work at Cornell University. He was associated with the Bureau of Dairy Industry, USDA,

for 27 years before his retirement in 1947. Prior to his association with the Bureau, he was with the Bureau of Mines in Denver and Pittsburgh. He was very active in civic affairs, serving as town commissioner and fire marshal of Cottage City. He was widely known for his research and survey of literature on ice cream. Surviving are Mrs. Leighton, one son, and one daughter.

Ivan McKellip, Professor of Animal Husbandry at Ohio State University for 32 years, died after a month-long illness in December, 1947. Prof. McKellip was graduated from the University of Nebraska, received his Master's degree from Cornell University and taught at Massachusetts State College and later at Purdue University. He was widely known as a judge of dairy animals and was most active in 4-H club work. He is survived by Mrs. McKellip, six sisters, and five brothers.

George Girrbach, Sault Ste. Marie, Michigan, was born in Minnesota in 1890. He died May 24, 1948, following an auto accident while en route home from attending a special session of the Michigan Senate, of which he was a member. Mr. Girrbach was graduated from the University of Minnesota and received his Master's degree from Michigan State College in 1927. He served as Dairy Extension Specialist at Michigan State College from 1924 to 1930, after which he was owner and manager of the Soo Creamery, Sault Ste. Marie, Michigan. He is survived by his wife, Mrs. Ethel Girrbach.

Helmar Rabild was born in Denmark on August 10, 1876, and was naturalized in 1905. He graduated from Denmark Agricultural College, was an instructor at Michigan Agricultural College from 1902 to 1906, and was associated with the Chesterfield Creamery in Michigan from 1902 to 1903. He was Deputy Dairy and Food Commissioner in Michigan from 1905 to 1907 and in charge of dairy extension at the Dairy Division of the USDA, Washington. He organized the first cow-testing association in the United States. At the time of his death, on January 1, 1948, he was president and manager of the Titusville Dairy Products Company, Titusville, Pennsylvania. He is survived by Mrs. Rabild.

Weston A. Price of Santa Monica, California, died on January 23, 1948. Information concerning his death was received too late for publication.

Respectfully submitted—H. P. DAVIS; E. O. HERREID; G. M. TROUT
Upon motion duly seconded, the report was accepted.

RESOLUTIONS COMMITTEE

WHEREAS: The University of Georgia through its administrative staffs and faculty has made available to the American Dairy Science Association in this its 43rd Annual Meeting all needed physical facilities for the meeting, and

WHEREAS: Every possible personal courtesy has been given to members of the Association for their enjoyment and entertainment,

Therefore, be it *Resolved*: That the American Dairy Science Association take this opportunity officially to extend its thanks and appreciation and hereby request the President of this Association to convey by letter this appreciation to President H. W. Caldwell and to Dean Paul W. Chapman and Prof. H. B. Henderson.

WHEREAS: The Borden Company has again offered its awards for outstanding research in dairy manufacturing and production,

Therefore, be it *Resolved*: That the American Dairy Science Association express to the Borden Company its sincere appreciation of this evidence of its continued interest in dairy research.

WHEREAS: The American Feed Manufacturers Association has seen fit to offer an award for outstanding research in the field of dairy cattle nutrition,

Therefore, be it *Resolved*: That the American Dairy Science Association express to the American Feed Manufacturers Association its sincere appreciation for their interest in and encouragement of research in dairy cattle nutrition.

WHEREAS: The Purebred Dairy Cattle Association has continued in its cooperation with the American Dairy Science Association in establishing uniform rules for the testing of dairy cattle, for the regulation of artificial breeding and other matters promoting uniformity and,

WHEREAS: The Purebred Dairy Cattle Association has established a Dairy Cattle Breeding Research Foundation for the purpose of encouraging and supporting research in this field, in cooperation with the various experiment stations,

Therefore, be it *Resolved*: That the American Dairy Science Association commend the Purebred Dairy Cattle Association for its efforts.

WHEREAS: Brucellosis is a serious disease of growing magnitude and concern to rural people, packing-house workers and veterinarians, and

WHEREAS: This disease affects cattle, swine and goats and causes great financial losses to the dairy and livestock industries,

Therefore, be it *Resolved*: That the American Dairy Science Association urges intensified brucellosis eradication activity throughout the country (at this time, particularly among cattle) with the eventual goal being brucellosis-free herds, counties and states.

Be it further *Resolved*: That all known and effective methods of control and eradication shall be used as individual state disease situations demand, and that additional research to find still better methods of control be further stimulated and encouraged to achieve the above objectives.

Be it further *Resolved*: That the Committee of the National Research Council under the chairmanship of W. W. Spink, appointed to investigate and study the relationships between brucellosis in humans and farm animals, be given the Association's encouragement and that the National Research

Council be urged to give the necessary aid and support for its continuance. A copy of this resolution shall be sent to the chairman of the Division of Biology and Agriculture of the National Research Council.

Respectfully submitted—S. L. TUCKY; W. A. KING; E. L. FOUTS

Upon motion duly seconded, the report was adopted.

REGISTRATION COMMITTEE

H. B. Henderson, University of Georgia, made the following report for the Registration Committee. Upon motion duly seconded it was accepted.

Alabama	9	Minnesota	16	Tennessee	14
Arizona	2	Mississippi	4	Texas	6
Arkansas	2	Missouri	11	Utah	1
California	4	Montana	1	Vermont	8
Connecticut	7	Nebraska	6	Virginia	8
Delaware	1	New Hampshire	4	Washington	4
Florida	12	New Jersey	11	West Virginia	5
Georgia	40	New Mexico	1	Wisconsin	37
Illinois	45	New York	40	Washington, D. C.	18
Indiana	7	North Carolina	18	Canada	5
Iowa	16	North Dakota	1		—
Kansas	7	Ohio	38	Total members	530
Kentucky	41	Oklahoma	5	Non-members	70
Louisiana	7	Oregon	4		—
Maine	1	Pennsylvania	14	Total	600
Maryland	17	Rhode Island	4	Women and children	356
Massachusetts	5	South Carolina	8		—
Michigan	15			Total attendance	956

Fordyce Ely moved and W. E. Peterson seconded that all action of the Executive Committee during the past year be approved.

MEETING OF THE EXECUTIVE COMMITTEE AMERICAN DAIRY SCIENCE ASSOCIATION

R. B. STOLTZ, *Secretary-Treasurer*

The Executive Committee transacted the following business:

Approved the Minutes of the past Annual Meeting.

Approved the Editor's Report.

Approved the Secretary-Treasurer's Report.

Approved the report of the Auditing Committee.

Approved the budget for 1948 amounting to \$35,500.

Received the report of the representative of the National Research Council.

Re-employed the Editor and Secretary for the ensuing year.

Voted unanimously to make A. A. Borland an Honorary Member.

Elected G. H. Wise as a member of the Journal Management Committee to serve for the ensuing three years.

Voted to not affiliate with the International Dairy Federation.

Adopted the Journal Management Committee report.

Approved the Resolutions Committee report.

Voted to make Robert S. Breed a life member of the Association.

Approved the motion that the budget include the cost of travel by first class in a public conveyance for each member of the Executive Committee not now provided for to attend the annual meeting.

Accepted the proposal by W. E. Petersen that the Association meeting be held at the University of Minnesota on June 20 to 22, 1949.

The Nominating Committee, consisting of G. H. Wilster, F. J. Doan and G. C. North, nominated the following officers in April: Vice-president, W. V. Price of Wisconsin, and G. M. Trout of Michigan; directors, E. E. Heizer, Wisconsin; H. B. Henderson of Georgia; P. R. Elliker of Oregon; and C. A. Iverson of Iowa.

The results of the election were announced on June 1 as follows: G. M. Trout, vice-president; H. B. Henderson and P. R. Elliker, directors.

THE AMERICAN DAIRY SCIENCE ASSOCIATION AWARDS

Athens, Georgia, June 16, 1948

H. B. Henderson, of the University of Georgia, acted as toastmaster at the barbecue and presented President P. H. Tracy, who installed the officers-elect as follows: W. E. Petersen, of Minnesota, was installed as President; G. M. Trout, of Michigan, as Vice-president; H. B. Henderson, of Georgia, and P. R. Elliker, of Oregon, as Directors.

Mr. Petersen, you are about to take over the responsibilities of President of the American Dairy Science Association. As President it will be your duty to be chairman of the Executive Board and submit to the Board for approval the nominations of members to fill vacancies that may occur among the elected officers of the Association. As President you shall appoint, without the approval of the Executive Board, the standing non-elective committees of the Association. With these obligations, privileges and responsibilities I now charge you with the honor of being President of the American Dairy Science Association with all the privileges, responsibilities and obligations pertaining thereto.

Mr. Trout, you are about to take over the responsibilities of Vice-president of the American Dairy Science Association. As Vice-president, it will be your duty to preside over the Executive Board in the absence of the President and assume other duties of the Executive Board. At the expiration of President Petersen's term, you will automatically become President of this Association. I now charge you with these duties.

Mr. Elliker and Mr. Henderson, you were elected to the Executive Board of the American Dairy Science Association. It is the duty of the Board



ANDREW ALLEN BORLAND

members to pass on all applications for the establishment of divisions, sections and student branches of the Association. You will have full control of the budget and general business of the Association and have title to all property and funds of the Association. You will be members of a Board that has all the rights and power vested in the by-laws of the Association. With these privileges, responsibilities and obligations you are now considered as members of the Executive Board of the American Dairy Science Association to serve a term of three years.

PRESENTATION OF ASSOCIATION AWARD

The toastmaster then introduced A. C. Ragsdale, chairman of the Association Honors Committee, who made the following citation:

Andrew Allen Borland was born at Sandy Lake, Pennsylvania, married Jesse E. Canon, and has two children, Gerald Canon and Margaret Eleanor. He taught in the public schools, Mercer County, Pennsylvania from 1898 to 1905, graduated from Pennsylvania State College in 1909, and received the Master's degree from the University of Wisconsin in 1910.

Mr. Borland served as assistant in Dairy Husbandry research, Pennsylvania State College from 1910 to 1911; as professor and head of the Animal and Dairy Husbandry Department, University of Vermont, from 1911 to 1915; as professor in charge of Dairy Husbandry extension, Pennsylvania State College, from 1915 to 1919; and as professor and head of the Dairy Husbandry Department, Pennsylvania State College, since 1919, from which position he retires June 30, 1948.

He served as a member of the Board of Education, Burlington, Vermont, from 1913 to 1915; and has been a member of the Board of Civic Planning, State College, Pennsylvania, since 1930; a trustee of the Westminster Foundation, State College, Pennsylvania, since 1939; vice-president of the Westminster Foundation, State College, Pennsylvania, since 1941; served as president of the Pennsylvania Dairymen's Association, 1925 to 1927; and as president of the College Feed Conference Board in 1931.

Mr. Borland served as Vice-president of the American Dairy Science Association, from 1920 to 1922; president of the American Dairy Science Association from 1922 to 1924; president of the Eastern Division of the American Dairy Science Association in 1925; a member of the Journal Management Committee, from 1932 to 1944; chairman of the Journal Management Committee from 1943 to 1944; contributing editor, *Pennsylvania Farmer*, since 1923; United States and Pennsylvania delegate to the 8th World's Dairy Congress, London, Reading, Edinburgh, Glasgow, 1928; and as U. S. judge, 4-H International Dairy Cattle Judging Contest, Kent, England, 1928.

He has been an elder in the Presbyterian Church for many years and active in church affairs. He is a member of the Masons, Phi Kappa Phi, Gamma Sigma Delta, Delta Sigma Rho, Alpha Zeta, and Delta Theta Sigma.

To select his most important contribution is a difficult task. In the field of research, he has given generously of his time as an advisor to his colleagues. We make special reference only to his leadership in the research project on "Input and Output Relationships in Milk Production" which he conducted in cooperation with the United States Department of Agriculture. He will be best remembered as an outstanding teacher and able administrator. As a teacher, he has been deeply interested in character building. His interest in people and their love for him has been evidenced in his extension activities, the deep affection of his students and his participation in church and civic affairs. The dairy industry owes him much for his outstanding leadership throughout the years.

It is with great pleasure and appreciation for his life of service that we present to Andrew Allen Borland this Certificate of Honorary Membership in the American Dairy Science Association.

PRESENTATION OF BORDEN AWARDS

B. E. Horrall, chairman of the Borden Award Committee for Manufacturing, was then introduced and made the following statements:

The recipient of the Borden Award in Dairy Manufactures for this year is one of the leading dairy scientists and has made many outstanding contributions in the field of Microbiology and Chemistry of dairy products. Most of his research has been in the fields of butter and cheese. He has made extensive studies on the surface taint of butter and isolated many organisms that cause the many off flavors in butter. He has made timely studies on surface bleach in butter, the cause of wood taint in storage butter and treatment of butter boxes for its prevention. His work on mold and yeast problems in butter has gained wide recognition. In his cheese research he has published many papers, some of which are studies on starter problems, effect of copper on lipase activity, development of rancid and unclean flavors, use of clarified milk in cheddar cheese manufacture, and development of lipolytic flavor defects in cheddar cheese. He developed the "Pink Test" for determining setting-time in cheddar cheese-making. These and many other of his research studies have definitely given the candidate an enviable record among the outstanding dairy scientists of the world.

He received his Bachelor's degree from Ontario Agricultural College and his Master's and Doctor's degrees from Massachusetts Agricultural College in 1913, 1919 and 1922, respectively. Besides these degrees, in which he majored in Bacteriology and minored in Chemistry, he spent some time in the U. S. Army Medical Corps, six months of which were spent at Yale taking Medical Bacteriology, after which he was posted at Army Base Hospitals at Houston, Texas, New Haven, Conn., and Chicago, Ill. During his stay at Massachusetts Agricultural College he also did much research work on black smut in canned lobsters.



EDGERTON GIBSON HOOD

It was the unanimous decision of the Borden Award Committee for Dairy Manufacturing that Dr. E. G. Hood be selected as the recipient for the Borden Award this year.

In the fall of 1923 Dr. Hood was appointed Chief of the Division of Dairy Research under the Dairy and Cold Storage Branch of the Dominion Department of Agriculture at Ottawa. He has been in charge of Dairy Research work since that time.

Since joining the Dominion Department of Agriculture, most of Dr. Hood's work has been directed towards the study of major problems confronting the dairy industry in Canada. Dr. Hood has become well known in dairy circles in Canada and the United States, not only for his research but also through his attendance at dairy conventions and his connection with the American Dairy Science Association, of which he served as director for several years.

Mr. Wentworth, it gives me great pleasure to present to you Dr. Egerton Gibson Hood as recipient for the 1948 Borden Award in Dairy Manufacturing.

Mr. Wentworth then presented to Dr. Hood a gold medal and a check for \$1,000.

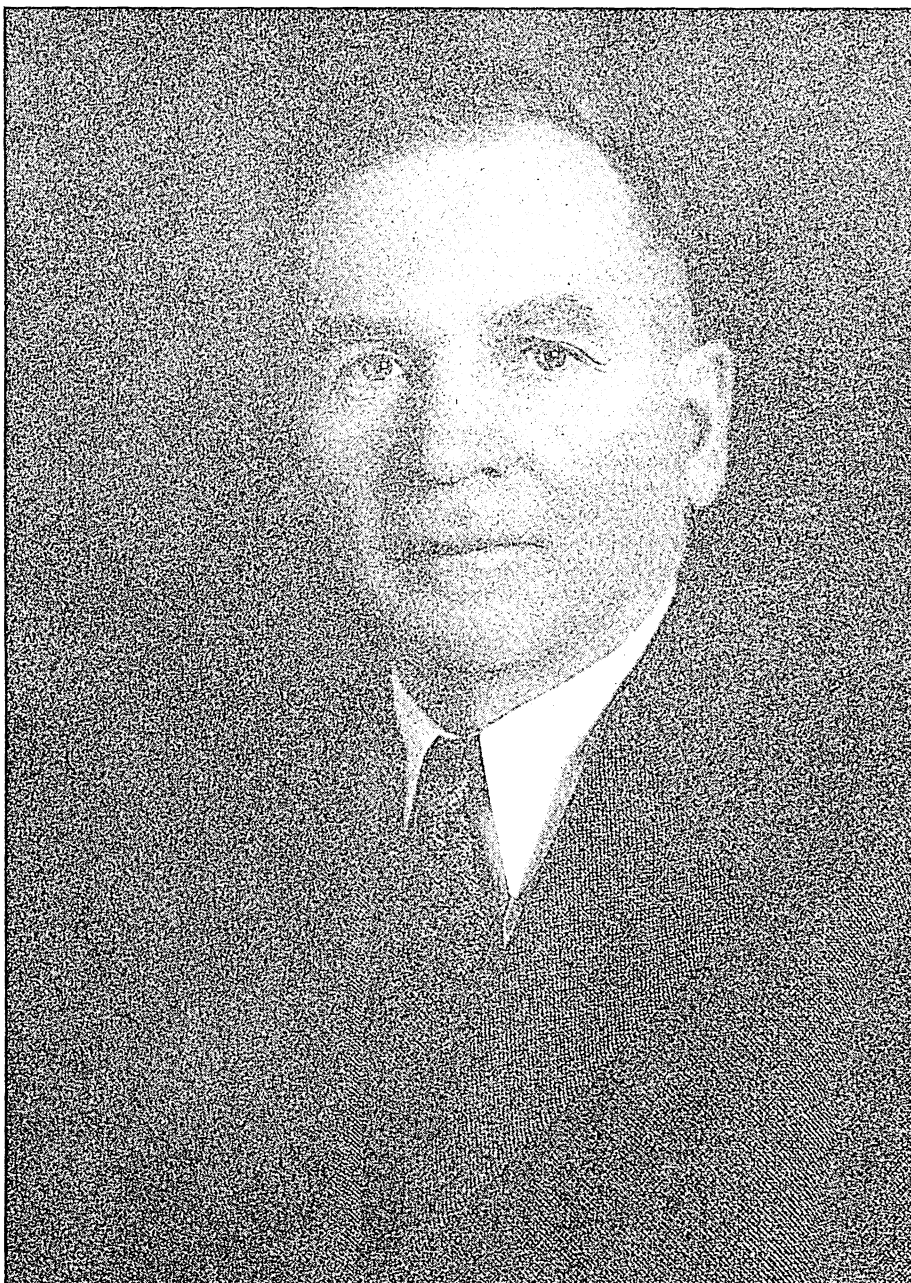
BORDEN AWARD IN DAIRY PRODUCTION

C. W. Turner, chairman of the Borden Award Committee for Production, then was introduced and made the following statement:

The recipient of the 1948 Borden Award in Production chosen by your committee has completed 40 years of intensely productive research at the University of Illinois. He is a native of that state, having been born at Crete, Illinois, in 1881. He was graduated with a B.S. degree from the University of Illinois in 1908 and received his Ph.D. degree from the University of Chicago in 1915.

His doctor's thesis, entitled "A Contribution to the Physiology of Lactation," published in 1915, not only set the pace for the high standards of excellence which he has maintained over the years in his publications but indicated the field of scientific interest which he has steadfastly pursued. He has been the modest leader in the United States of the scientific approach to the problems of milk secretion. He was one of the first to apply physiological methods to the problem of the "let down" of milk. His study of the quantity of milk present in the udder of the cow at milking time followed. Other early classic studies included the "relation between percentage fat content and yield of milk," "feed cost of milk production as affected by the percentage fat content of the milk," and "relative rates of secretion of various milk constituents."

These investigations culminated in the report on the "energy basis for measuring milk yields in dairy cows." The formula proposed for the



W. L. GAINES

equalization of milk yield upon the basis of the energy content of the milk has gained universal acceptance throughout the world. Fat-corrected milk (FCM) is standard practice in dairy cattle nutrition work as well as in milk secretion studies.

To the problem of the factors influencing the lactation curve of dairy cattle was applied not only a keen understanding of lactational physiology, but also mathematical and inventive genius as well. Slide rules and intricate mechanical equipment were designed to assist in the enormous task of fitting equations to the individual cow's lactation curve included in the extensive studies conducted over a period of years. Not only did his work point to the importance of persistency in the lactational performance of dairy cattle but the extent of the influence of pregnancy upon milk yield was accurately determined.

Measures of the efficiency of dairy cattle and the relation of body weight thereto next engaged the attention of this investigator. More recently the problem of the energy-size basis of measuring milk yield has been explored. Some 46 technical papers and bulletins have come from his pen. However, a mere enumeration of numbers of publications cannot indicate the numerous contributions of a truly fundamental character. One fellow investigator has said that "his work on the energy aspects of milk constituents is known around the world and quoted in writings in other lands perhaps more widely than that of any other American worker in dairy science."

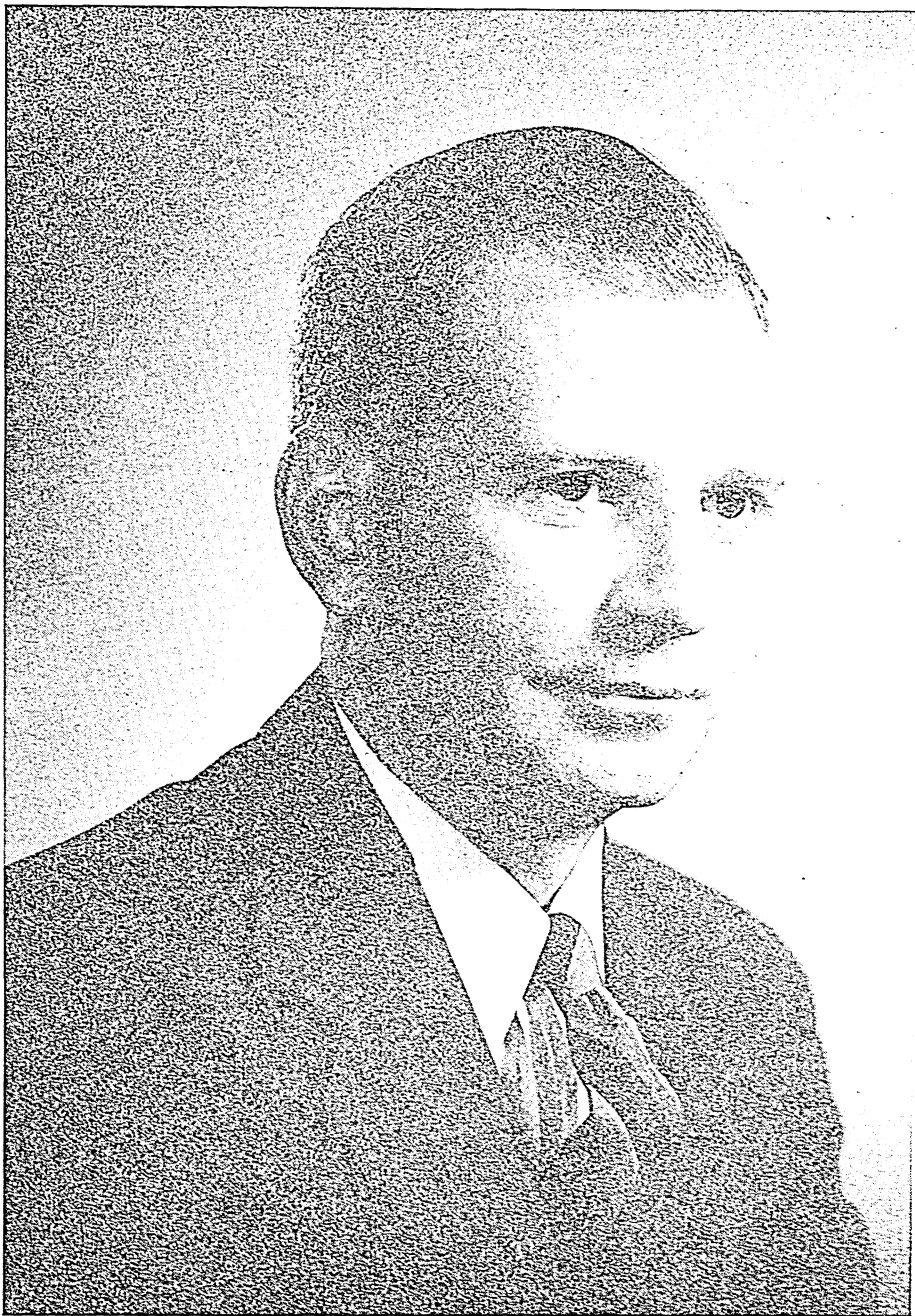
One phase of his character less easy to evaluate has been his influence upon younger investigators in exemplifying the proper attitude, spirit and methods of the research worker. It has been said of him that "he has been cautious, skeptical in the best scientific sense of the word, always eager to get the actual evidence concerning any point under discussion, industrious, full of eager scientific curiosity, patient and gentle but firm in discussions and controversies with younger men. Many a younger worker has gone away from scientific meetings which he attended, having his thoughts clarified, any feeling of personality in the controversy removed, and with renewed eagerness to do some really sound work in advancing still farther the frontiers of knowledge in dairy science."

On behalf of the committee on the Borden Award in Dairy Production, it is a very great personal pleasure and an honor to present Dr. W. L. Gaines, Professor and Chief in Milk Production of the Illinois College of Agriculture, to receive the Award.

Mr. Wentworth then presented Dr. Gaines a gold medal and a check for \$1,000.

R. B. Becker, acting chairman of the Award Committee for the American Feed Manufacturers' Association, then was introduced and made the following statement:

Early in the year, the American Feed Manufacturers' Association de-



GEORGE HERMAN WISE

sired to encourage and recognize superior original research in dairy cattle nutrition and asked the American Dairy Science Association to establish rules, evaluate published work, and designate the outstanding contributions. Publications during 1946 and 1947 were eligible for the 1948 award. Four points were considered in evaluating the work, namely: original research, proper presentation, value in dairy cattle nutrition, and the possibility of practical use to the dairy cattle industry.

Nominations by many workers pointed out several leading investigations. The award committee also searched recent technical journals, proceedings, and research publications for other meritorious contributions. The selection of a series of seven publications was unanimous, based on outstanding investigations conducted at two experiment stations under the leadership of one worker. This original work scored highest. The award is for contributions on effect of prepartum diet of the cow on vitamin A and tocopherol contents of colostrum and early milk, upon vitamin storage in the newborn calf, and upon the physiology of gastric digestion in the young calf. The investigations were begun at Kansas State College and completed at Iowa State College. The man who led these investigations and receives the award is Dr. George H. Wise.

P. R. Record, vice-chairman of the Nutritional Council of the American Feed Manufacturers' Association, then presented Dr. Wise with a check for \$1,000.

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THE EFFECT OF PYRIDIUM, PENICILLIN, FURACIN, AND PHENOXETHOL UPON THE LIVABILITY OF SPERMATOOZOA AND UPON THE CONTROL OF BACTERIA IN DILUTED BULL SEMEN^{1, 2}

R. H. FOOTE AND G. W. SALISBURY³

Laboratory of Animal Breeding and Artificial Insemination, Department of Animal Husbandry, Cornell University, Ithaca, New York

The same types of organisms associated with vaginal infections and abortion in cows sometimes are found in the semen of bulls. A majority of the sulfonamides tested on semen samples (7) markedly reduced bacterial growth at levels which also increased the livability of the spermatozoa. Whether or not this improvement in spermatozoan livability which accompanied bacteriological control was a phenomenon distinctly associated with the sulfonamide compounds was not established.

The bacteriostatic and/or bactericidal effects of pyridium (8), penicillin (2), furacin (4, 5), and phenoxethol (3, 9) have been demonstrated on a wide variety of organisms. Almquist *et al.* (1) reported that penicillin controlled bacterial growth in diluted bull semen at levels which had no significant effect on livability or fertility. Phillips and Lardy (11) noted that "certain antibiotics, including penicillin, were not harmful to sperm motility". So far as is known to the authors, no studies have been reported on the effects of adding pyridium, furacin, and phenoxethol to diluted bull semen. The present paper is a report of the comparative effect of pyridium, furacin, phenoxethol, two penicillins, and sulfanilamide on bacterial growth and spermatozoan livability.

EXPERIMENTAL

The design and methods used in conducting the experiments reported in this paper were the same as those described previously (7). Solutions

Received for publication February 18, 1948.

¹ The data published in this paper have been taken from a thesis presented by the senior author to the Graduate School, Cornell University, in partial fulfillment of the requirements for the degree of Master of Science in Agriculture, 1947.

² The authors are indebted to Merck and Company, Inc., for the pyridium; to the Eaton Laboratories, Inc., of the Norwich Pharmaceutical Company for the furacin; to the Heyden Chemical Corporation for one of the commercial penicillins, and to A. H. Allard for technical assistance in these experiments.

³ Now at the University of Illinois.

of pyridium, furacin, and phenoxethol were prepared in the same manner as were the sulfonamide solutions(7). Since penicillin comes in sterile vials and is unstable to heat, varying concentrations of penicillin solutions were prepared aseptically just prior to use in each experiment.

Eighteen first ejaculates, 13 second ejaculates, and 2 third ejaculates were used in the experiment. These had an average initial motility of 66 per cent motile spermatozoa which were moving at an average rate of 3.2, where 4.0 is considered maximum. The mean concentration was 1,180,000 spermatozoa per mm.³, and the mean methylene blue reduction time was 6.7 minutes.

Pyridium. The azo dye, pyridium, was soluble only to the extent of about 5 mg. per 100 ml. of citrate diluent. Apparently as a consequence of the low concentration, pyridium had very little effect on the livability of the spermatozoa or on the control of bacterial growth.

Five milligrams per 100 ml. of pyridium added to five semen samples stored in citrate diluent at 37.5° C. and eight semen samples stored in citrate-phosphate diluent at 20° C. had no observable effect, either beneficial or harmful, on the livability of the spermatozoa and failed to reduce bacterial growth as compared to the untreated controls.

Sulfanilamide, the positive control, was significantly superior statistically in maintaining livability of the spermatozoa. Also, it was highly effective in inhibiting bacterial growth.

Penicillin. Two commercial penicillins, *A* and *B*, each were added to four separate ejaculates stored at 20° C. in citrate-phosphate diluent at the rates of 0, 63, 125, 250, 375, and 500 Oxford Units of penicillin per ml. of diluted semen. All additions of penicillin *A* were toxic to the spermatozoa, whereas additions of penicillin *B* were not. Both penicillins were equally effective in reducing bacterial growth and were more effective than the 300 mg. per 100 ml. of sulfanilamide in this respect.

A subsequent experiment was conducted in yolk-citrate diluent at 5° C. By adding penicillin at the rate of 200, 400, 600 and 800 Oxford Units per ml. of diluted semen, penicillin *A* again was found to be toxic to the spermatozoa and penicillin *B* was not. The results are shown in table 1. As in the previous report (7), the mean motility values shown in the table were calculated from the individual observations made at specified intervals until the spermatozoa in a majority of the treatments were dead.

In maintaining the motility of the spermatozoa, sulfanilamide was found to be significantly superior, statistically, to all levels of penicillin tested. Where the treatments were not toxic, only sulfanilamide slowed the rate of motility. Whether commercial penicillin *A* was composed chiefly of a penicillin different from commercial penicillin *B* or whether it contained a substance which was toxic to the spermatozoa (12) was not determined.

In the ten samples used for the motility estimation, a study of bacterial growth after 4 and 8 days of storage revealed that bacterial contamination was small in all treatments. Neither the sulfanilamide nor the two penicillins were particularly effective in controlling the organisms which survived at 5° C. Countable plates were obtained from five of the seven ejaculates treated with penicillin B, the results of which are included in table 1. Similar results were recorded for penicillin A.

Furacin. It quickly was established that large quantities of furacin added to spermatozoa stored in the citrate-phosphate buffer at 20° C. were deleterious to the spermatozoa. Lower levels of furacin were studied on the semen obtained from four different bulls. At the four lowest levels

TABLE 1

The effect of additions of penicillin A and B to yolk-citrate upon the livability of spermatozoa and bacterial growth

Treatment	No. of ejaculates	Storage at 5° C.	Controls		Oxford units of penicillin/ml.				
			SA ^a 300 mg. %	0	200	400	600	800	
(days)			% motile spermatozoa						
Penicillin A	3	16	37.5	35.0	31.0	27.6	21.5	15.2	
Penicillin B	7	16	42.4	39.4	40.0	39.4	39.1	36.3	
			Rate of motility						
Penicillin A	3	16	1.5	1.6	1.1	0.9	0.7	0.4	
Penicillin B	7	16	1.4	1.6	1.5	1.5	1.6	1.5	
			Bacterial count per ml.						
Penicillin A	5	4	18,500	27,000	19,000	14,000	17,500	10,000	
Penicillin B	5	8	14,000	25,000	16,000	11,500	13,000	10,000	

^a SA = Sulfanilamide.

of furacin studied (0, 0.5, 1.0 and 2.0 mg. per 100 ml. of diluent), the mean motilities for the experiment were 42, 41, 34 and 27 per cent, respectively, the last two means being significantly different, statistically, from the first two means. Therefore, furacin was considered to be spermicidal.

Furacin proved to be an effective bacterial antagonist at the higher concentrations, but levels which were not harmful to livability of the spermatozoa were only moderately effective in controlling bacterial growth. When furacin was added to two ejaculates at the rate of 0, 0.5, 1.0, 2.0, 5.0 and 10.0 mg. per 100 ml. of diluent, the average plate count after 72 hours of storage was 81,000,000, 24,800,000, 2,760,000, 106,000, 7,000 and 650 organisms per ml., respectively. The two controls containing 300 mg. per cent of sulfanilamide averaged 2,670,000 organisms per ml. of diluted semen.

TABLE 2

The effect of adding phenoxethol to citrate-phosphate diluent at 20° C. upon livability of spermatozoa and bacterial growth
(Mean of 3 ejaculates)

Agent	Conc. (%)	% motile	Rate of motility	Bacterial Count/ml. ^a
Phenoxethol	0	52.0	2.6	44,000
	0.125	20.0	0.6	8,000
	0.25	0.0	0.0	2,000
	0.50	0.0	0.0	1,500
	1.00	0.0	0.0	900
	2.00	0.0	0.0	100
Sulfanilamide	300 mg.%	53.3	2.2	22,000

^a Platings made after 24 hr.

Phenoxethol. None of the compounds previously mentioned exerted any marked bacteriostatic effect on *Pseudomonas pyocyaneus*. The reports in the literature (3, 9) that phenoxethol was effective against this organism were substantiated in this laboratory using a pure culture of pseudomonas organisms isolated from a sample of semen.

Varying concentrations of phenoxethol were added to three semen samples and were found to be highly spermicidal. In the same study, after 24 hours of storage, subsamples were taken for plating. The data are presented in table 2. While all levels of phenoxethol tested partially inhibited bacterial growth. *Pseudomonas pyocyaneus* continued to thrive at the 0.125 and 0.25 per cent levels of this drug.

Numbers of bacteria present in fresh semen. Throughout the course of these studies sufficient data were accumulated to make possible a comparative study of the numbers of bacteria present in the first and second ejaculates collected within a few minutes of each other. The data for 47 such comparisons are summarized in table 3.

TABLE 3

A comparison of the number of organisms found in first and second ejaculates collected from bulls of high and low fertility

Bulls	Ejaculates	Range of bacteria in thousands per ml.										
		1-4	5-9	10-24	25-49	50-99	100-249	250-499	500-999	1,000-2,499	2,500-4,999	Over 5,000
		No. of ejaculates in each group										
NYABC ^a	1st	2	4	3	3	6	4	2	0	1 ^b	0	1 ^b
	2nd	4	3	3	6	4	4	0	1 ^b	1 ^b	0	0
Vitamin A deficient	1st	1	0	0	0	0	4	3	7	4	2	0
	2nd	1	0	0	0	1	3	9	4	3	0	0

^a NYABC = New York Artificial Breeders Cooperative, Inc.

^b Bulls of low fertility.

Twenty-four of the 26 bulls used by the New York Artificial Breeders Cooperative, Inc., were highly fertile during the period in which the collections were made. The other two bulls were disposed of shortly thereafter because of their low fertility records. The vitamin A-deficient bulls, from which 21 paired ejaculates were collected, were not used for breeding purposes. However, they were believed to be of relatively low fertility. These results are in agreement with Gunsalus *et al.* (10), who found more bacteria in the first than in subsequent ejaculates. Furthermore, the semen from bulls of known high fertility contained fewer organisms than semen from the other bulls studied.

DISCUSSION

The data presented in this paper show that a number of compounds, chemically unrelated to the sulfonamides, effectively controlled bacterial growth. With the exception of penicillin, it was impossible to establish drug levels which were consistent with bacterial control and at the same time were not detrimental to the spermatozoa. This is in rather sharp contrast with the previous report (7) in which nine out of 12 sulfonamides in bacteriostatic or bactericidal concentrations exerted a favorable influence upon the livability of the sperm cells. This favorable influence appears to be associated with the "slowing down" of the spermatozoa noted when certain sulfonamides were present in the diluent. These phenomena were not observed when the compounds reported herein were added to the different diluents.

Penicillin, while exerting no apparent beneficial effect on the spermatozoa, was slightly superior to sulfanilamide in controlling bacterial growth. However, the decided differences in toxicity observed between the two penicillins studied present a practical problem should it become desirable to take advantage of the bactericidal properties of penicillin. The use of synthetic penicillins (6) which recently have become available offers a possible approach to the problem.

SUMMARY AND CONCLUSIONS

1. The possible usefulness of pyridium was limited by its low solubility. Maximum attainable concentrations produced no noticeable effects on spermatozoan livability or on bacterial growth.

2. Two commercial penicillins were equally effective in controlling bacterial growth and were slightly superior to sulfanilamide in this respect. Neither penicillin proved to be beneficial to the spermatozoa, and one in particular was toxic even when present in small amounts.

3. Furacin and phenoxethol proved to be highly bactericidal, and at the same time were spermicidal at most of the levels tested. Organisms of the pseudomonas group, especially, were resistant to the lower concentrations studied.

4. Throughout these experiments only sulfanilamide, the positive control, actually improved the livability of the spermatozoa.

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THE EFFECT OF SULFONAMIDES UPON THE LIVABILITY OF SPERMATOOZOA AND UPON THE CONTROL OF BACTERIA IN DILUTED BULL SEMEN¹

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The possible influence of bacteria on the results of metabolic studies and on the results of fertility in artificial insemination has been under investigation at this laboratory for some time. With the present trend of shipping the diluted semen over greater distances and inseminating animals after the diluted semen has undergone longer periods of storage, other factors, in addition to the original quality, may become increasingly important in maintaining high levels of fertility. One such factor is bacteriological control.

In view of the fact (6) that sulfonamides, with proper variation of the factors influencing sulfonamide action, inhibit the metabolism of cells of nearly every variety, and that certain sulfonamides increased the livability (8, 12) and fertility (13) of bovine spermatozoa, several sulfonamides were selected for study. Furthermore, contrary to earlier findings (3, 7, 10, 17), several investigators (5, 15, 18) reported that sulfonamides administered in human therapy did not impair the livability of the spermatozoa. Likewise *in vivo* and *in vitro* studies with rams (1) and rodents (4, 9, 11, 14) failed to demonstrate that the sulfonamides were deleterious to spermatogenesis or to the survival of the spermatozoa. Since levels of sulfonamides consistent with bacteriological control and optimum for the survival of the sperm cells had been ascertained for sulfanilamide only (8), it was logical that investigations were undertaken to study various sulfonamides in these respects.

EXPERIMENTAL

Materials and methods. Because of the large number of sulfonamides tested and the limited quantity of semen in each ejaculate, it was impossible to compare the sulfonamides with each other by adding all of them to the same series of ejaculates. Therefore, each experiment included a positive control containing sulfanilamide at the established optimum level of 300 mg. of sulfanilamide per 100 ml. of diluted semen (8), and a negative control

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containing no sulfonamide. By means of these positive and negative controls it was possible to compare the relative effects of each treatment employed.

Since length of life in storage was considered the best measure of the effect of each sulfonamide on livability of the spermatozoa, microscopic examinations to determine the percentage of motile spermatozoa and the rate of motility were made until the spermatozoa in a majority of the treatments were dead. The motility values recorded at each observation were used in calculating the mean motility values presented in the accompanying tables. Tubes containing the stored samples were coded, and at the time of each microscopic examination they were randomized to prevent the introduction of bias on the part of the investigator due to knowledge of the treatment in the particular sample under observation.

Three series of experiments were conducted. In the series I experiments, bacterial growth was measured by changes in optical density of the stored semen samples as described by Knodt and Salisbury (8) and by the plate count method. Thereafter, only the plate count method was used, for which veal infusion agar served as the culture medium. Plates were counted after 48 and 96 hours of incubation at 37.5° C. Only the total counts recorded at 96 hours are presented in this paper, inasmuch as colonies of the slow-growing diphtheroids frequently were too small to count at 48 hours.

The sulfonamides, or sulfas as they sometimes are called, selected for use were sulfathiazole (ST), sulfapyridine (SP), sulfamerazine (SM), sulfaguanidine (SG), sulfaquinoxaline (SQX), sodium sulfadiazine (NaSD), sodium sulfathiazole (NaST), carboxysulfathiazole (Carb.ST), sodium sulfamerazine (NaSM), sodium sulfamethazine (NaSMT), N¹-benzoylsulfanilamide (N¹-BSA), sulfasuxidine (SS), and sulfanilamide (SA) as a control.

All sulfonamide solutions were prepared by adding the desired quantities of each sulfonamide to the basic diluter used. These solutions then were autoclaved simultaneously at 15 lb. pressure for 20 minutes.

The 23 first ejaculates, 11 second ejaculates, and 2 third ejaculates used had a mean initial motility of 66 per cent motile spermatozoa moving at a rate of 3.3, where 4.0 is considered maximum, a mean concentration of 1,069,000 spermatozoa per mm.³; and a mean methylene blue reduction time of 6.9 minutes.

RESULTS

Series I. Semen diluted with citrate-sulfonamide diluter and stored at 37.5° C. In this series of experiments the basic diluter consisted of 3.6 g. of sodium citrate dihydrate made up to 100 ml. of water distilled in glass. One part of fresh semen was added to nine parts of the citrate-sul-

fonamide diluter and stored at 37.5° C. Six sulfonamides, at 0, 50, and 100 per cent of the maximum solubility of each drug as established for this diluter, were studied initially. Motility and optical density observations were made at 0, 6, 12, 24, and 48 hours of storage, and subsamples were taken for bacterial counts at 24 and 48 hours of storage.

After five ejaculates had been so treated, this study was terminated, for in nearly all cases most of the spermatozoa were dead after less than 12 hours of storage. Because of the adverse effect at 37.5° C. of the diluting medium upon the livability of the spermatozoa, it was impossible to measure any effect that the particular sulfonamides tested may have had on the livability of the spermatozoa.

The optical density of the stored samples increased appreciably. How-

TABLE 1
*Bacterial growth in diluted semen stored with different sulfonamides
at 37.5° C. as measured by plate counts
(Mean of 3 ejaculates)*

Drug	Conc. (mg. %)	Rank at 24 hr.	Plate counts (1,000's/ml.) after storage for:		Rank at 48 hr.
			24 hr.	48 hr.	
SA	300	1	252	18,300	2
ST	200	2	401	13,000	1
ST	100	3	9,170	26,200	3
SA	150	4	10,300	68,500	5
SM	90	5	20,200	64,000	4
SG	90	6	34,400	144,000	8
SQX	50	7	40,600	139,000	7
SP	100	8	50,700	97,000	6
SQX	25	9	66,800	178,000	9
SG	45	10	72,800	323,000	12
SM	45	11	80,100	197,000	10
SP	50	12	102,000	221,000	11
O	13	793,000	4,970,000	13

ever, the formation of the sulfonamide crystals in several of the treatments as the acidity increased in the stored samples interfered with the measurement of optical density changes associated with bacterial growth. Consequently only bacterial growth as measured by the plate count method will be reported. These data are summarized in table 1. The drugs are ranked on the basis of the effectiveness with which each controlled bacterial growth. Sulfanilamide, as well as several other sulfonamides, exerted a pronounced bacteriostatic effect. As a rule, bacterial growth was inversely proportional to the amount of the sulfonamide present, irrespective of the particular one used.

Series II. Semen diluted with citrate-phosphate-sulfonamide diluter and stored at 20° C. The poor livability encountered in the experiments

reported in series I was circumvented by reducing the temperature to 20° C. and by developing a more suitable diluter. The new diluter, C-P, consisting of 0.2 g. potassium dihydrogen phosphate, 0.29 g. anhydrous disodium phosphate, and 1.8 g. of sodium citrate dihydrate made up to 100 ml. water distilled in glass, was found to preserve the motility of spermatozoa for several days at 20° C. The pH of this diluent, following autoclaving, was

TABLE 2
The effect of sulfonamide additions to C-P diluter upon the percentage of motile spermatozoa
(Means for the entire experiment)

No. of ejaculates	Name of drug	Maximum sol. of each drug	% motile spermatozoa				Controls	
			Levels of the drugs expressed as % of maximum solubility				0	SA 300 mg. %
			12.5	25	50	100		
		(mg. %)						
3	ST	200	37.7	43.3	24.4	37.0	45.0
5	ST	27.4	33.2	Toxic	29.8	33.0
8	ST	36.6	Toxic	33.7	35.8
3	SP	100	37.7	32.0	27.7	37.0	45.0
5	SP	29.0	23.6	Toxic	29.8	33.0
8	SP	27.9	Toxic	33.7	35.8
3	SM	90	44.1	40.7	37.8	37.0	45.0
5	SM	32.0	28.7	N.T. ^a	29.8	33.0
8	SM	32.1	N.T.	33.7	35.8
3	SG	90	44.7	40.7	37.8	37.0	45.0
5	SG	29.2	31.0	N.T.	29.8	33.0
8	SG	34.2	N.T.	33.7	35.8
3	SQX	50	40.7	40.1	42.7	37.0	45.0
5	SQX	29.7	31.4	N.T.	29.8	33.0
8	SQX	34.4	N.T.	33.7	35.8
9	NaSD	250	34.4	34.6	31.2	30.8	33.3
3	NaST	500	17.1	13.4	9.1	23.1	22.3
4	NaST	34.0	34.0	28.8	Toxic	37.5	37.8
6	NaST	24.9	Toxic	Toxic	31.2	31.3
4	Carb.ST	600 ^b	28.8	31.8	27.1	20.5	27.3	27.0
4	NaSM	500	29.8	26.8	Crystals	27.3	27.0
5	NaSMT	500	31.8	36.2	Crystals	30.6	28.8
4	N1-BSA	500 ^b	26.0	22.8	5.6	26.8	28.8
4	SS	600 ^b	25.8	25.0	25.8	19.8	27.3	27.0

^a N.T.=not tested as previous tests indicated toxicity.

^b arbitrary maximum as these drugs were highly soluble.

6.90 as compared to the autoclaved citrate used previously which had a pH of about 7.75.

Motility observations were made on the semen samples at 8, 24 and 48 hours, and every 24 hours of storage thereafter, until most of the spermatozoa were dead. Usually progressive movement was observed in the diluted semen samples for at least 6 days under these conditions. In other respects, these experiments were conducted in the same manner as were those in series I.

The percentage of motile spermatozoa and the rate of motility were observed on the stored ejaculates from 15 bulls. Since it was found that with two exceptions the relative rates of motility paralleled the relative percentages of motile spermatozoa, "rate" observations will be discussed only briefly. Means for the percentage of motile spermatozoa are shown in table 2.

At the higher concentrations (100 per cent of maximum solubilities of each drug), all of the more soluble sulfonamides were toxic. When a particular level of a sulfa was established as toxic or at least not beneficial to the spermatozoa, lower concentrations of the drug were studied. These

TABLE 3

Bacterial growth in diluted semen stored with different sulfonamides at 20° C. as measured by plate counts

(Mean of 4 ejaculates; platings made after 24 hr. of storage)

Name of drug	Drug conc. (mg. %)	Rank	Plate counts (1,000's/ml.)
ST	100	1	36 ^a
SA	300	2	52
SA	150	3	99
ST	50	4	163
NaSD	500 ^b	5	200
NaSD	250 ^b	6	252
SP	50	7	300
SP	25	8	309
SM	45	9	313
SM	22.5	10	334
SQX	25	11	417
SQX	12.5	12	547
SG	45	13	570
SG	22.5	14	1,637
O	15	5,900

^a Some colonies hidden by spreading organisms.

^b Sulfa crystals observed.

results are included in the table. Nine of the 12 drugs tested were superior to the negative controls in preserving the life of the spermatozoa, while three of the 12 drugs equaled or excelled sulfanilamide in this respect. Of these three—sodium sulfadiazine, sodium sulfamethazine, and carboxy-sulfathiazole—only the latter two were significantly superior to sulfanilamide (significant, respectively, at the 5.0 and 1.0 per cent levels of probability). The apparent superiority of sodium sulfamethazine and carboxysulfathiazole over sulfanilamide may be due to the fact that for some unknown reason sulfanilamide in the control failed to benefit the spermatozoa.

The additions of sulfanilamide consistently decreased the rate of movement of the spermatozoa. While the difference between the negative and positive controls was not great, it was significant statistically. N¹-benzoyl-

sulfanilamide markedly reduced the rate of movement and almost completely immobilized the spermatozoa long before they died. No particular pattern was established by the other sulfonamides.

Plate counts were made on all semen samples stored for the motility observations, but several of the samples contained large numbers of spreading organisms which prevented accurate counting. The number of bacteria in the stored semen samples from which countable plates were obtained is reported in tables 3 and 4. As was expected, the organisms

TABLE 4
*Bacterial growth in diluted semen stored with different sulfonamides
at 20° C. as measured by plate counts*
(Mean of 2 ejaculates; platings made after 24 hr. of storage)

Name of drug	Drug conc.	Rank	Plate counts (1,000's/ml.)
	(mg. %)		
N ¹ -BSA	500	1	13
SA	300	2	15
NaST	250	3	20
N ¹ -BSA	250	4	31
NaST	500	5	33
Carb.ST	150	6	38
N ¹ -BSA	125	6	38
NaST	125	8	40
NaSMT	500 ^a	9	46
NaSMT	125	10	51
NaSM	500 ^a	11	58
NaSM	250	12	64
SS	300	13	81
NaSMT	250	14	97
Carb.ST	300	15	103
NaSM	125	16	114
Carb.ST	75	17	169
SS	75	18	176
SS	150	19	255
O	20	303

^a Sulfa crystals observed.

multiplied rapidly at the storage temperature of 20° C. in the negative controls, although much less rapidly than at 37.5° C. Here again several sulfonamides markedly inhibited bacterial growth. Increasing the concentrations of the sodium salts of several sulfonamides beyond the point where crystallization occurred did not increase materially the bacteriostatic effect of the drugs. In one experiment, 0.25, 0.50, 1.0, 2.0, 4.0, 6.0, and 8.0 g., respectively, of NaSD per 100 ml. were added to diluted semen. Additions beyond the saturation point (0.25 g.) did not increase the effectiveness with which bacteria were controlled.

Series III. Semen diluted with yolk-citrate-sulfonamide diluter and stored at 5° C. Under routine conditions in artificial insemination, buffered egg yolk is used as the diluent, and the diluted semen is stored at 5° C.

Therefore, it was desirable to test, under these conditions, concentrations of the drugs which reduced bacterial growth and/or improved spermatozoan livability in the citrate-phosphate diluter at 20° C. Sulfathiazole, NaSD, NaSMT, and Carb.ST were shown to be equal or superior to sulfanilamide in at least one of these respects.

To accommodate the four drugs tested at four different levels of each drug plus a positive and negative control for each drug, five semen samples were split into 24 equal subsamples. One part of semen was added to nine parts of diluter. Motility examinations were made every 2 days until a majority of the treatments showed no motile spermatozoa. The mean

TABLE 5

The effect of ST, NaSD, NaSMT, and Carb.ST upon the livability of spermatozoa in yolk-citrate stored at 5° C.

(Mean of 5 ejaculates, and a total of 1200 observations)

Drug tested		Controls					
		No sulfa			SA 300 mg. %		
ST	mg. % in diluter	50	75	100	125
	% motile sperm.	36.4	35.3	33.9	30.6	35.0	37.6
NaSD	mg. % in diluter	150	200	250	300
	% motile sperm.	33.7	32.4	33.0	31.9	35.8	37.3
NaSMT	mg. % in diluter	100	150	200	250
	% motile sperm.	33.1	33.4	29.9	26.9	35.6	38.0
Carb.ST	mg. % in diluter	50	100	150	200
	% motile sperm.	39.0	40.4	36.6	31.8	36.1	38.3

motility for each of the treatments is presented in table 5. Appropriate statistical analyses revealed that the differences observed between the negative control and both carboxysulfathiazole and sulfanilamide were significant at the 5 per cent level of probability.

More than 1,000 plate counts³ were made after 4 and 8 days of storage to determine the number of organisms present in the diluted semen used for the motility observations. Most of the organisms in the five ejaculates studied did not live well at the storage temperature of 5° C. Consequently, little differentiation due to treatment was observed. At 4 days the counts

³ By washing the eggs according to Bryant and Sharp (2), wearing sterilized rubber gloves, and using all sterilized equipment, it was possible to obtain the egg yolk used in the diluent free, or almost completely free, from bacteria.

ranged from 11,000 to 43,000 organisms per ml. and at 8 days from 13,800 to 109,000 organisms per ml., with the low count being obtained from a semen sample to which sulfanilamide had been added and the high count from a sample to which no bacterial inhibitor had been added. Standard laboratory tests for the identification of *Pseudomonas pyocyaneus* showed a small percentage of pseudomonas organisms in the fresh semen of three out of the five ejaculates. After 4 and 8 days of storage, the pseudomonas organisms made up a large proportion of the total number of organisms present.

DISCUSSION

The experimental evidence presented in this paper demonstrates that all of the 12 sulfonamide preparations investigated were effective in reducing the rate of bacterial growth. Plate counts made on diluted semen stored for 24 hours at 20 and at 37.5° C. showed that sulfanilamide, sulfathiazole, and some of the other sulfonamides generally prevented the bacterial population from increasing to more than about four times that at zero time. The bacterial population increased 800 times over the population at zero time in diluted semen containing no sulfonamide.

At 5° C. and in the presence of egg yolk, none of the sulfonamides tested markedly decreased the growth of the organisms which were able to survive the storage temperature of 5° C. A majority of these surviving organisms were of the pseudomonas group. At the temperatures studied, the pseudomonas group was not inhibited markedly by levels of the sulfonamides which were not harmful to the spermatozoa.

The evidence presented shows that large doses of the sulfonamides may be toxic to the spermatozoa. However, with three exceptions, levels were established for each sulfonamide which improved the livability of the spermatozoa and, at the same time, were consistent with bacteriological control. Even at 20° C., satisfactory motility of the spermatozoa was maintained for many days in the presence of several of the sulfonamides.

While sulfanilamide fairly consistently increased the duration of motility, it consistently decreased the rate of motility. In view of the report of Knodt and Salisbury (8) that oxygen consumption by spermatozoa is depressed in the presence of sulfanilamide, this slowing down of the spermatozoa is believed to result from a depressing effect by sulfanilamide on cellular metabolism.

SUMMARY AND CONCLUSIONS

1. Twelve sulfonamides were added to bull semen diluted with citrate-phosphate and stored at 20° C. At the level of each drug determined to be optimum for spermatozoan survival, nine of the 12 drugs increased the livability of the spermatozoa over that observed when no sulfonamide was

added to the diluent. Of these nine, only two sulfonamides, sodium sulfamethazine and carboxysulfathiazole, were significantly superior to sulfanilamide in maintaining motility of the sperm cells, but they were inferior in controlling bacterial growth. Sulfanilamide slightly decreased the rate of motility, and N¹-benzoylsulfanilamide exerted a similar but more pronounced effect. No consistent pattern was established by the other sulfonamides.

2. At 20 and at 37.5° C. all of the sulfonamides were effective in reducing bacterial growth at levels which were not harmful to the spermatozoa. *Pseudomonas pyocyaneus* was not inhibited at these levels.

3. At 5° C. the sulfonamides were only slightly effective in controlling bacterial growth because the pseudomonas group of organisms predominated in the bacterial flora surviving at this temperature.

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BREEDING BEHAVIOR, SPERMATOGENESIS, AND SEMEN PRODUCTION OF MATURE DAIRY BULLS FED RATIONS LOW IN CAROTENE

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The present emphasis on extending the use of the desirably proved dairy sire in artificial breeding has focused attention on the need for more information concerning the nutrition of the mature sire, particularly with respect to the role of nutrition in semen production and fertility. In both, spermatogenesis is of primary concern.

The importance of vitamin A and carotene in developing and maintaining the normal germinal epithelium and the breeding ability of young bulls has been demonstrated by Sutton *et al.* (14), Jones *et al.* (7), Hodgson *et al.* (6) and Erb *et al.* (4, 5). Regeneration of the germinal epithelium following vitamin A and carotene therapy has been observed (4, 6). Similar studies with mature bulls have not been reported.

Roughages are the principal source of carotene for bulls of breeding age. If allowed pasture, mature bulls might be expected to build up body stores of vitamin A sufficient to carry them through periods of relatively low carotene intakes. On the other hand, the continued use of low-carotene roughages might deplete body stores to the extent that breeding ability and spermatogenic activity would be impaired. This possibility raises the question as to how long mature bulls will continue to produce normal semen and remain free from clinical manifestations of vitamin A deficiency when fed a low-carotene ration of concentrates and poor quality roughage. An investigation was undertaken in August, 1945, to measure the changes in semen production and to study the development of clinical manifestations of vitamin A deficiency in mature dairy bulls.

EXPERIMENTAL PROCEDURE

The experimental plan was to feed six relatively mature breeding bulls a low-carotene concentrate mixture along with poor quality roughage for a period of at least 1 year, unless serious clinical symptoms developed earlier. No deficiencies were apparent by the end of that period of time. Therefore, the roughage component of the ration was changed to dried beet pulp.

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and three of the bulls were fed a supplement of carotene in oil, sufficient to furnish approximately 30 mg. of carotene per 100 lb. of body weight per day. The remaining three bulls received no supplement of carotene. The 30-mg. level of carotene supplementation represented approximately 5.5 times the level for maintenance of beef bulls of equivalent weight as recommended by the Committee on Animal Nutrition of the National Research Council (11).

It was not possible at the beginning of the experiment in August, 1945, to secure six bulls that were over 5 years of age. However, semen had been collected from the two youngest bulls at irregular intervals for approximately 1 year before the initiation of the experiment. The breed and age of the bulls at the beginning of the experiment were as follows: *N*, a Holstein, age 4 years, 7 months; *C*, a Brown Swiss, age 2 years, 10 months; *J*, a Guernsey, age 8 years, 6 months; *M*, a Guernsey, age 6 years, 4 months;

TABLE 1
Carotene content of the roughages fed

Periods during which a particular roughage was fed	Kind of roughage	Carotene content (mg./lb.)
1 to 6, incl.	Hay	4.24
7 to 10, incl.	Hay	1.27
11	Hay	1.70
12	Wheat straw	1.70
13	Wheat and oat straw	0.56
14 to 16, incl.	Oat straw	1.80
17 to 21, incl.	Dried beet pulp ^a

^a Not analyzed; assumed to have no carotene or vitamin A potency.

K, a Guernsey, age 8 years, 7 months; *I*, a Guernsey, age 2 years, 9 months.

The feed allowance for individual bulls was established on the basis of their individual initial body weights, their appetites and their general tendency to lose or gain in body weight. Changes in the feeding schedule were infrequent. Roughage feeding was scheduled to supply approximately 60 per cent of the T.D.N. and the concentrate mixture the remaining 40 per cent.

The concentrate mixture fed consisted of 40 lb. of ground oats, 10 lb. of ground barley, 12 lb. of ground wheat, 17 lb. of wheat bran, 8 lb. of soybean oil meal, 4 lb. of linseed oil meal, 7 lb. of cane molasses, 1 lb. of steamed bone meal and 1 lb. of common salt. This mixture analyzed 0.22 mg. of carotene per pound. The calculated digestible protein content was 13.8 per cent and the T.D.N. content 71.8 per cent.

The carotene content of the roughages fed and the daily feed intakes of the bulls are shown in tables 1 and 2, respectively.

The response criteria and the methods for their measurement were as follows:

Body weights. In addition to initial and final weights on each bull, monthly average weights were secured representing one, two or three daily weights, all of which were taken within a 7-day interval, usually at the beginning of the monthly period.

Blood plasma carotene and vitamin A. The carotene and vitamin A content of blood plasma from each bull was determined by the procedure of Kimble (8) on samples obtained on 3 successive days each month.

Semen production. From each bull two ejaculates were collected at 10-day intervals by means of an artificial vagina. The volume of semen per ejaculate, per cent motile spermatozoa, total spermatozoa per milliliter of semen and the per cent abnormal spermatozoa in each ejaculate were measured according to the standard procedures of this laboratory (10, 13, 15).

TABLE 2
Daily feed intakes of the bulls by periods

Periods	Kind of feed	lb. per day fed each bull					
		Bull N	Bull C	Bull J	Bull M	Bull K	Bull I
1 to 7, incl.	Concentrates	8	8	8	8	8	8
	Hay	18	18	18	18	18	12
8 to 16, incl.	Concentrates	8	8	8	7	8	8
	Hay or straw	18	18	18	14	18	12
17 to 21, incl.	Concentrates	8.4	8.2	6.2	6.2 ^a	7.2	6.2
	Dried beet pulp	12.4	12.0	9.2	9.2	10.8	9.2
	Carotene ^b (mg.)	(576)	(0)	(404)	(0)	(476)	(0)

^a During period 21, the scheduled amounts for bull M were reduced one-half.

^b Carotene in oil, analyzing 35,371 γ per g. of oil, was fed daily to furnish the intakes indicated. Values in parentheses are mg. per bull per day.

Clinical manifestations of vitamin A deficiency. The time intervals, in days, between the beginning of the feeding of the dried beet pulp, with and without the carotene-in-oil supplement, and the onset of night blindness, papillary edema, incoordination, edema of the extremities, loss of libido and breeding ability were noted.

Histological examination of testes. Testis tissue, obtained at the time the bulls were slaughtered, was fixed in Allen's PFA₃ fluid (modified Bowin's) (9), within 30 to 40 minutes after death. Microscopic examination of the seminiferous tubules for the extent of degeneration of the germinal epithelium was made on 7- μ sections which had been stained with an iodine-ripened hematoxylin (2) and counterstained with eosin.

Liver carotene and vitamin A. The whole livers were removed at death, stored at 40° F. and ground and sampled within 12 hours after death. A 10- to 25-g. composite sample was used for the extraction and determination of carotene and vitamin A according to the method of Davies (3).

Feed analyses. Chemical analyses of samples of the concentrates and roughages fed were made according to the methods of the A.O.A.C. (1). Carotene in the feeds as fed was estimated by the method of Nelson *et al.* (12).

The carotene content of the "carotene in oil" was determined colorimetrically by dissolving the oil in petroleum ether and measuring the density of the yellow color in a Lumitron colorimeter with a 440 m μ filter, the colorimeter previously having been standardized against pure β -carotene.

Analysis of data. In order to summarize the voluminous observations made during the experiment, the data were consolidated into twenty-one 30-day periods, each period representing three 10-day semen collection intervals. Within each of the 30-day periods, the observations made of each of the following criteria—body weights, blood plasma carotene and vitamin A, volume of semen per ejaculate, per cent motile spermatozoa, total spermatozoa per milliliter of semen and per cent abnormal spermatozoa per ejaculate—were summed and the average obtained. These averages then were considered as single observations in all subsequent analyses of the data. All semen data averages were based on the combined first and second ejaculates. All bulls finished the first 16 periods but during the following 5 periods one pair of bulls, *K* and *I*, was slaughtered because of the development of severe avitaminosis A by bull *I*.

RESULTS AND DISCUSSION

The 30-day period averages for body weight, estimated daily intakes of carotene, and plasma carotene and vitamin A of each bull are presented in tables 3 and 4. During the first 16 periods (480 days), the bulls generally increased in body weight (table 3). During the final 5 periods (150 days) two of the bulls, *M* and *I*, which were not receiving the carotene supplement, showed slight losses in body weight with the onset of avitaminosis A. In contrast, bull *C* gained in weight during these latter periods.

In table 4 the plasma carotene and vitamin A values reveal some apparent deviations from a smooth curve, but, in general, these variations can be explained by the carotene intakes. The plasma vitamin A level was low (2 to 3 γ per 100 ml. plasma) for bulls *C* and *M* before pronounced deficiency symptoms were evident.

The averages for each bull, by 30-day periods, for the criteria of semen production and spermatozoan characteristics are shown in tables 5 and 6. No consistent changes in relative semen volume were demonstrated during the first 16 months of the experiment (table 5). The per cent of motile spermatozoa (table 6) showed marked decreases for bulls *C* and *I* during the 11th, 12th, and 13th periods. These decreases in motility were accompanied by equally marked increases in the per cent of abnormal spermatozoa (table 5). These concurrent changes suggest that both bulls may have been ap-

TABLE 3
Average body weights and estimated daily intakes of carotene

30-day period	Body weight in lb.										Mg. carotene fed per day									
	Bulls					Bulls					Bulls					Bulls				
	N	J	K	C	M	I	N	J	K	C	M	I	N	J	K	C	M	I		
Aug. '45	Ration: Poor quality roughage, hay or straw, plus low-carotene concentrate mixture																			
1	1776	1246	1535	1551	1323	1083	78.1	78.1	78.1	78.1	61.0	52.7	78.1	78.1	78.1	78.1	61.0	52.7		
2	1824	1269	1553	1609	1354	1135	78.1	78.1	78.1	78.1	61.0	52.7	78.1	78.1	78.1	78.1	61.0	52.7		
3	1854	1281	1541	1628	1369	1188	78.1	78.1	78.1	78.1	61.0	52.7	78.1	78.1	78.1	78.1	61.0	52.7		
4	1887	1337	1581	1682	1362	1211	78.1	78.1	78.1	78.1	61.0	52.7	78.1	78.1	78.1	78.1	61.0	52.7		
5	1872	1350	1584	1663	1379	1224	78.1	78.1	78.1	78.1	61.0	52.7	78.1	78.1	78.1	78.1	61.0	52.7		
6 ^a	78.1	78.1	78.1	78.1	61.0	52.7	78.1	78.1	78.1	78.1	61.0	52.7		
7	24.7	24.7	24.7	24.7	19.4	17.0	24.7	24.7	24.7	24.7	19.4	17.0		
8	1866	1338	1603	1723	1307	1257	24.7	24.7	24.7	24.7	19.4	17.0	24.7	24.7	24.7	24.7	19.4	17.0		
9	1847	1298	1536	1690	1226	1229	24.7	24.7	24.7	24.7	19.4	17.0	24.7	24.7	24.7	24.7	19.4	17.0		
10	1801	1263	1495	1628	1198	1199	24.7	24.7	24.7	24.7	19.4	17.0	24.7	24.7	24.7	24.7	19.4	17.0		
11	1792	1249	1422	1578	1167	1213	32.4	32.4	32.4	32.4	25.4	22.2	32.4	32.4	32.4	32.4	25.4	22.2		
12	1832	1265	1423	1624	1195	1241	32.5	32.5	32.5	32.5	25.5	22.3	32.5	32.5	32.5	32.5	25.5	22.3		
13	11.9	11.9	11.9	11.9	9.5	8.5	11.9	11.9	11.9	11.9	9.5	8.5		
14	1903	1328	1522	1715	1247	1308	34.3	34.3	34.3	34.3	26.9	23.5	34.3	34.3	34.3	34.3	26.9	23.5		
15	1897	1315	1555	1751	1289	1363	34.3	34.3	34.3	34.3	26.9	23.5	34.3	34.3	34.3	34.3	26.9	23.5		
16	1924	1348	1588	1836	1346	1369	34.3	34.3	34.3	34.3	26.9	23.5	34.3	34.3	34.3	34.3	26.9	23.5		
Dec. '46	Ration: Dried beet pulp, low carotene concentrate, plus carotene-in-oil supplement for bulls N, J, and K																			
17	1822	1265	1520	1732	1252	1357	578	405	478	1.8	1.4	1.4	578	405	478	1.8	1.4	1.4		
18	1825	1294	1527	1760	1269	1343	578	405	478	1.8	1.4	1.4	578	405	478	1.8	1.4	1.4		
19	1875	1300	1485	1840	1268	1287	578	405	478	1.8	1.4	1.4	578	405	478	1.8	1.4	1.4		
20	1958	1306	Slaugh-tered	1885	1296	Slaugh-tered	578	405	478	1.8	1.4	1.4	578	405	478	1.8	1.4	1.4		
21	2024	1326	2-27-47	1927	1253	2-27-47	578	405	478	1.8	1.4	1.4	578	405	478	1.8	1.4	1.4		

^a..... no observations made.

proaching a deficiency state. In the case of bull *I*, the average number of spermatozoa per milliliter of semen (table 5) decreased during these same periods.

During the last 5 months, when bulls *C*, *M* and *I* were on the beet pulp and low-carotene concentrate mixture without the carotene supplement, semen volume again was characterized by considerable variation without a definite trend accompanying the low plasma vitamin A values and the onset of clinical symptoms. At the same time, the per cent of motile spermatozoa decreased and the per cent of abnormal spermatozoa increased as these bulls developed advanced vitamin A deficiency (table 6).

The concentration of spermatozoa per milliliter of semen for the combined first and second ejaculates varied considerably up to the time the last samples were collected. Of those bulls receiving no carotene supplement, bull *I* exhibited an increase in spermatozoan concentration while bulls *C* and *M* exhibited decreases in concentration just prior to the time they were unable to mount.

The bulls receiving the carotene supplement exhibited sufficient variation in the several semen and spermatozoan characteristics that no particular improvement was attributed to the increased carotene intake.

Figure 1 shows photomicrographs of histological sections of the seminiferous tubules of the right testis of each bull. These photomicrographs are by pairs of bulls and show the contrasting conditions of the tubules accompanying the presence and absence of supplemental carotene in the basal ration. Bull *C* exhibited the most advanced degeneration of the germinal epithelium, followed by bull *I* and bull *M* in order. All exhibited a similar pattern of degeneration, namely, few spermatogonia, relatively small numbers of spermatocytes and immature spermatids. The seminiferous tubules of the bulls receiving the carotene supplement apparently were normal, with the exception of bull *K*, and showed numerous dividing spermatogonia; spermatocytes, maturing spermatids, and many spermatozoa in the lumen of the tubules.

It should be pointed out that the testes sections of bull *K* showed occasional tubules which were characterized by almost complete degeneration of the germinal epithelium and the absence of spermatids and spermatozoa in the lumen. Whether or not these represented unrepaired tubules arising from the feeding regime prior to the carotene supplementation is, in the absence of biopsy data, mere speculation.

Table 7 presents for comparison the observations on the onset of the clinical symptoms in terms of days after the change in roughage from hay and straw to beet pulp for those bulls not receiving the carotene supplement. Considerable variation occurred between bulls in the rapidity with which they developed incoordination, edema of the extremities and night blindness. This variation, undoubtedly, was a reflection of their respective

TABLE 5
Average semen volume per ejaculate and numbers of spermatozoa per ml.
(Averages represent first and second ejaculates)

30-day period	Semen volume per ejaculation in ml.										Numbers of spermatozoa per ml. $\times 10^6$									
	Bulls					Bulls					Bulls					Bulls				
	N	J	K	C	M	I	N	J	K	C	M	I	N	J	K	C	M	I	N	J
Aug. '45	Ration: Poor quality roughage, hay or straw, plus low-carotene concentrate mixture																			
1	4.3	5.6	3.9	4.1	6.3	3.8	1.21	1.23	0.60	1.09	1.16	0.89	1.21	1.23	0.60	1.09	1.16	0.89	1.21	1.23
2	6.4	5.5	6.5	4.3	5.9	3.3	1.04	1.17	0.92	0.96	1.08	0.82	1.04	1.17	0.92	0.96	1.08	0.82	1.04	1.17
3	4.4	4.2	5.8	4.3	6.8	3.1	1.45	1.11	0.78	1.54	1.13	1.05	1.45	1.11	0.78	1.54	1.13	1.05	1.45	1.11
4	3.7	5.8	7.2	3.9	8.3	2.6	1.48	1.20	0.77	1.39	1.06	0.90	1.48	1.20	0.77	1.39	1.06	0.90	1.48	1.20
5	4.5	5.2	6.6	3.5	6.9	2.5	0.99	0.99	0.76	0.81	1.31	0.81	0.99	0.99	0.76	0.81	1.31	0.81	0.99	0.99
6	3.5	6.1	7.0	5.5	6.2	2.2	1.11	1.28	0.59	1.18	1.15	0.81	1.11	1.28	0.59	1.18	1.15	0.81	1.11	1.28
7	4.3	4.6	6.0	4.6	6.6	2.5	0.79	1.14	0.59	0.88	1.12	0.61	0.79	1.14	0.59	0.88	1.12	0.61	0.79	1.14
8	3.6	4.7	5.2	5.9	6.3	2.7	1.12	0.76	0.69	1.13	0.86	0.64	1.12	0.76	0.69	1.13	0.86	0.64	1.12	0.76
9	3.9	4.4	4.4	4.8	5.5	2.1	1.21	0.90	0.52	0.74	1.08	0.49	1.21	0.90	0.52	0.74	1.08	0.49	1.21	0.90
10	3.5	3.8	5.5	4.2	5.2	3.0	0.90	1.17	1.10	0.98	0.83	0.48	0.90	1.17	1.10	0.98	0.83	0.48	0.90	1.17
11	3.6	5.4 ^a	5.4	4.3	1.6	1.04	1.12 ^a	1.40	1.06	0.38	1.04	1.12 ^a	1.40	1.06	0.38	1.04	1.12
12	2.9	2.7 ^a	4.0	7.1	3.4	0.92	0.80	0.62	1.08	1.28	0.50	0.92	0.80	0.62	1.08	1.28	0.50	0.92	0.80
13	2.7	6.2	4.6	4.1	5.9	3.4	0.71	1.16	0.97	0.97	0.68	0.64	0.71	1.16	0.97	0.97	0.68	0.64	0.71	1.16
14	3.3	4.8	2.9	4.9	5.8	3.0	0.80	0.84	0.52	1.07	0.83	0.64	0.80	0.84	0.52	1.07	0.83	0.64	0.80	0.84
15	2.9	5.5	7.5	4.6	5.9	2.8	1.00	0.94	0.74	0.77	0.93	0.44	1.00	0.94	0.74	0.77	0.93	0.44	1.00	0.94
16	4.2	5.4	8.0	4.5	7.7	3.0	0.79	1.12	0.56	1.03	1.04	0.71	0.79	1.12	0.56	1.03	1.04	0.71	0.79	1.12
Dec. '46	Ration: Dried beet pulp, low carotene concentrate, plus carotene-in-oil supplement for bulls N, J, and K																			
17	4.0	6.6	6.0	4.5	6.2	3.6	0.89	0.73	0.86	0.70	1.05	0.56	0.89	0.73	0.86	0.70	1.05	0.56	0.89	0.73
18	5.9	6.9	7.1	5.6	5.7	3.7	1.28	0.76	0.90	0.97	1.27	0.97	1.28	0.76	0.90	0.97	1.27	0.97	1.28	0.76
19	6.8	6.8	6.5 ^c	5.5	6.1 ^b	1.22	1.14	0.64 ^c	0.79	1.22 ^b	1.22	1.14	0.64 ^c	0.79	1.22 ^b	1.22	1.14
20	6.0	7.9	Slaughtered	5.6	5.0	Slaughtered	1.17	0.87	Slaughtered	0.61	1.07	Slaughtered	1.17	0.87	Slaughtered	0.61	1.07	Slaughtered	1.17	0.87
21	7.4	6.7	2-27-47	3.9	3.1	2-27-47	1.14	0.83	2-27-47	0.21	0.73	2-27-47	1.14	0.83	2-27-47	0.21	0.73	2-27-47	1.14	0.83

^a No semen collected.

^b Unable to mount.

^c Represents only one collection during the period.

TABLE 6
Average percentages of motile and of morphologically abnormal spermatozoa
(Averages represent first and second ejaculates)

30-day period	% motile spermatozoa												% morphologically abnormal spermatozoa											
	Bulls						Bulls						Bulls											
	N	J	K	C	M	I	N	J	K	C	M	I	N	J	K	C	M	I						
Aug. '45	Ration: Poor quality roughage, hay or straw, plus low-carotene concentrate mixture																							
1	58	58	68	62	59	63	5	12	13	26	15	21												
2	61	53	53	56	54	43	11	10	10	20	19	22												
3	55	53	43	39	59	42	9	13	11	17	16	32												
4	59	65	67	67	56	61	9	17	14	22	21	40												
5	62	58	48	55	48	56	11	13	16	12	20	29												
6	60	60	51	53	55	53	16	13	17	15	19	33												
7	65	63	73	63	64	59	12	14	16	15	18	32												
8	53	56	55	57	62	58	9	14	15	15	19	30												
9	66	59	52	62	60	60	11	11	15	10	19	29												
10	66	54	60	50	53	44	12	13	15	11	22	47												
11	66	63 ^a	19	61	33	10	10 ^a	66	20	85												
12	65	57 ^a	38	54	34	8	12 ^a	27	22	55												
13	62	48	49	47	58	49	9	16	13	19	17	25												
14	53	50	43	58	41	52	10	15	16	7	18	30												
15	53	53	55	53	50	44	9	14	10	6	16	27												
16	50	46	45	53	43	47	7	12	12	10	15	24												
Dec. '46	Ration: Dried beet pulp, low carotene concentrate, plus carotene-in-oil supplement for bulls N, J, and K																							
17	60	48	45	57	51	31	7	9	9	8	11	25												
18	65	47	53	44	44	29	6	8	9	12	12	24												
19	52	46	30 ^c	40	44 ^b	6	8	8	20	13 ^b												
20	56	53	Slaugh-tered	36	36	Slaugh-tered	4	9	Slaugh-tered	31	26	Slaugh-tered												
21	67	45	2-27-47	45	20	2-27-47	3	12	12	64	68	2-27-47												

^aNo semen collected.^bUnable to mount.^cRepresents only one collection during the period.

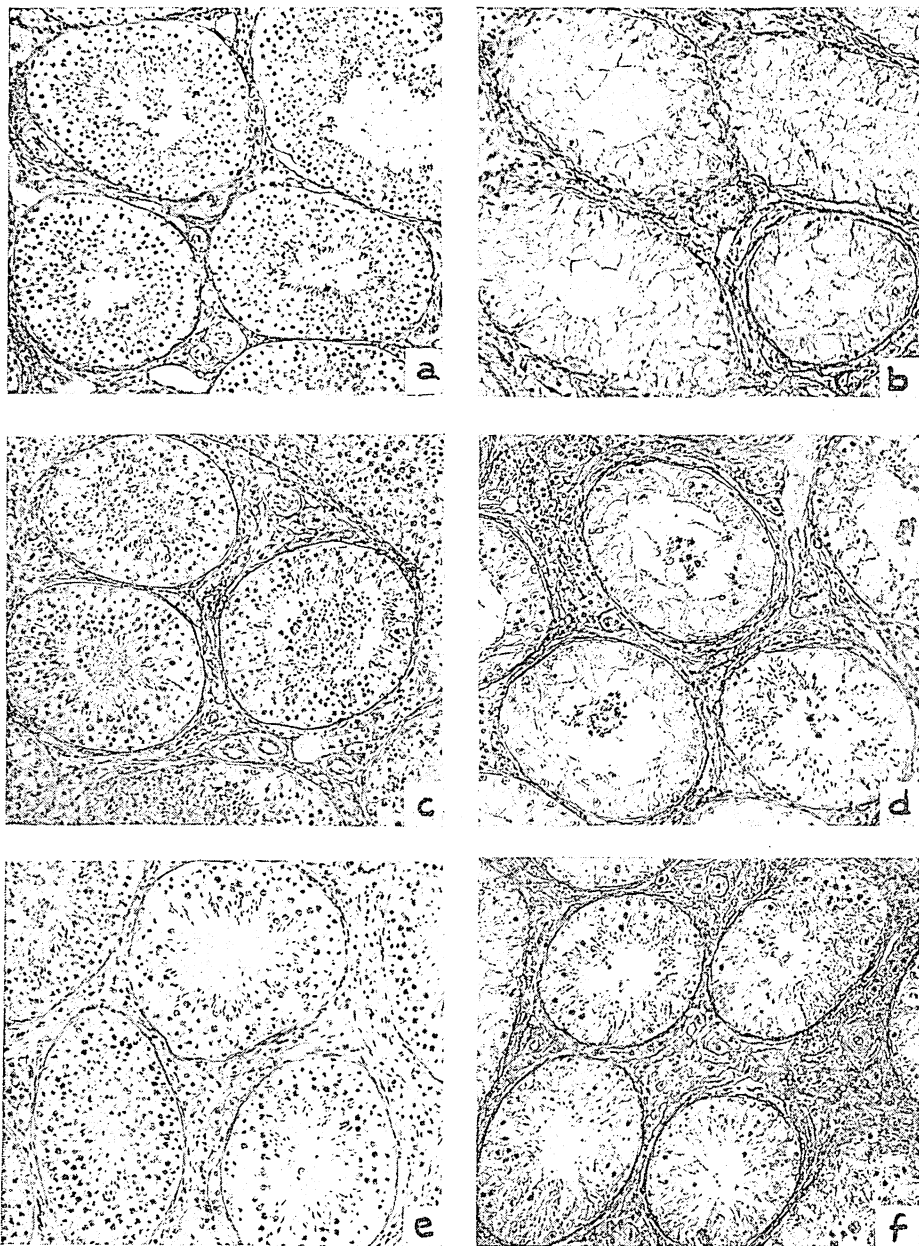


FIG. 1. Photomicrographs of seminiferous tubules of the right testis of each bull at time of slaughter. ($\times 100$.) a, c and e—bulls *N*, *K* and *J* after 151, 73 and 151 days, respectively, on the ration supplemented with carotene. b, d and f—bulls *C*, *I* and *M* after 151, 73 and 151 days, respectively, on the ration unsupplemented with carotene.

TABLE 7

Number of days bulls were fed deficient ration before onset of first clinical symptoms of vitamin A deficiency

Bull	Off feed	First signs of incoordination	Unable to mount	Extreme incoordination	Stiffness in legs	Marked edema of knees, hocks, and ankles	Night blindness ^a
C ^b	90	140	140	130-140	150	150 ^c
M	38 ^d	120	150 ^e	150	150 ^f	150 ^g
I	38	40	50-60	70	60	70 ^f

^a All bulls on the unsupplemented ration showed varying degrees of characteristic bleaching of the tapetum lucidum.

^b Bull C was never off feed.

^c Hemorrhagic areas about the papilla of right eye.

^d Bull M was off and on feed several times during the course of the feeding of the deficient diet. Scouring was also a frequent accompaniment of his erratic appetite.

^e Bull M was sufficiently incoordinated that he undoubtedly would not have been able to mount in another 10 days.

^f Condition not in evidence at completion of experiment.

^g It was questionable whether this bull was actually night blind.

body stores of carotene and vitamin A and their rates of depletion on the experimental rations employed.

In general, it would appear that incoordination and loss of the ability to mount, without the loss of libido, are the earliest signs of vitamin A deficiency in mature breeding bulls and occur before any marked impairment in semen production is manifested.

The liver stores of carotene and vitamin A at the time of slaughter are shown in table 8. The values reflect clearly the rations fed and the breeds used.

From the evidence of this investigation and the reports of others, the

TABLE 8

Carotene and vitamin A content of the fresh livers at the time of completion of experiment for individual bulls

Bull	Breed	No. of days between date of change of ration and date of slaughter	γ per g. fresh liver	
			Carotene	Vitamin A
Ration: Dried beet pulp, low carotene concentrate mixture, plus daily supplement of carotene-in-oil				
N	Holstien	151	438	778
J	Guernsey	151	1335	273
K	Guernsey	73	873	73
Ration: Dried beet pulp and low-carotene concentrate mixture				
C	Brown Swiss	151	28	12
M	Guernsey	151	48	13
I	Guernsey	73	47	5

authors believe that, while typical clinical manifestations of vitamin A deficiency and degeneration of the germinal epithelium can be produced in mature dairy bulls, the feeding of poor quality dry roughages, presumably low in carotene, for extended periods of time will not likely cause noticeable impairment of semen production before the onset of the clinical manifestations of A deficiency.

SUMMARY AND CONCLUSIONS

Six dairy bulls were fed dry roughages low in carotene and a concentrate deficient in carotene and vitamin A for a period of 16 months without inducing clinical manifestations of vitamin A deficiency or noticeable impairment of semen production.

Subsequent changes in the roughage component of the ration of three of the bulls from hay and/or straw to dried beet pulp plus the same concentrate mixture resulted in the development of incoordination, edema of the extremities, papillary hemorrhage, a gradual increase in the per cent of abnormal spermatozoa and a decrease in the per cent of motile spermatozoa, but no consistent change in the number of spermatozoa per milliliter of semen within a period of 40 to 120 days. These three bulls were unable to mount but still retained an unusual amount of libido. This inability to mount was manifested before the changes in semen production.

Typical patterns of degeneration of the germinal epithelium of the seminiferous tubules were found in the three bulls on the carotene-deficient rations. There were few spermatogonia, spermatocytes, spermatids or maturing spermatozoa in the lumen of the tubules.

While supplementing the carotene-deficient ration of the other three bulls with carotene in oil prevented the degeneration of the germinal epithelium which characterized those bulls not receiving the supplement of carotene in oil, it produced no consistent changes in semen production which reasonably could be attributed to the carotene.

Liver carotene and vitamin A levels of the carotene-deficient bulls were of the order of 30 to 50 γ and 5 to 13 γ per g. of fresh liver, respectively.

ACKNOWLEDGMENT

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FURTHER STUDIES OF THE NUTRITIVE VALUE OF BUTTERFAT FRACTIONS¹

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It was reported (4) that butterfat collected in September, 1945, was fractionated by crystallization from acetone at -4° C. and yielded two fractions; a liquid one (II) which promoted growth in rats at a superior rate as compared with butterfat or corn oil, and a solid one (I) which allowed a significantly inferior growth rate. This phenomenon was not observed to the same degree in later trials when samples of butter collected in subsequent months, including September, 1946, were used. Since the pasture of the summer of 1947 was considered as generally better than that of 1946, it was thought advisable to repeat the same fractionation procedure using the late summer butterfat from cows on this better pasture.

Fractionation of milk fat by cold crystallization also has been reported by Jack *et al.* In their early work (5) they did not obtain any one fraction with a growth promoting action superior to that of the whole milk fat. In a recent paper (6), they report that by the use of purified solvents they obtained a milk fat fraction which produced a significantly better growth in young rats than the original milk fat and also greater growth than supported by the other milk fat fractions. They attributed their first results to the effect of impurities in the solvents on the fat.

EXPERIMENTAL

The September, 1947, butter obtained from the University dairy was fractionated in the same manner described in the first paper of this series (1), again using purified acetone as the solvent, and at a temperature of -5° C. Fraction I had a melting point of 42° C., and an iodine number of 21, fraction II, a melting point of 7° C., and an iodine number of 51.

The fractions, as well as the untreated butterfat and corn oil, were used as the separate sources of fat in the diets fed to four groups of six rats each. Male weanling rats of the Sprague-Dawley strain weighing 40–50 g. were placed on the diets, the basic composition of which is shown in table 1.

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² Government of India Research Fellow.

TABLE 1
Composition of the diets

Components	28% Diet	10% Diet
Fat ^a	28	10
Sucrose	48	66
Casein ^b	20	20
Salts IV ^c	4	4
Vitamin supplement mg. per 100 g. diet		
Thiamine		0.2
Riboflavin		0.3
Pyridoxine		0.3
Ca pantothenate		1.5
Choline hydrochloride		150
β-Carotene ^d		0.56
α-Tocopherol		2.24
Calciferol ^e
2-Methol-1,4-naphthoquinone		0.21

^a Butterfat or corn oil or one of the fractions.

^b Extracted for three 2-hour periods with boiling 95% alcohol.

^c Phillips, P. H., and Hart, E. B., J. Biol. Chem., 109: 657. 1936.

^d 90% β-carotene and 10% α-carotene.

^e Crystalline irradiated ergosterol.

The fat level of the diet in this experiment was 28 per cent. The rats were housed in individual metal cages with raised screen bottoms, watered and fed daily *ad libitum* and weighed once weekly. Consumption records were kept during the fifth week, but since the growth of the groups on the corn oil and fraction I diets was irregular during this week, only the efficiency of the butterfat and fraction II diets was calculated.

Table 2 gives the gain in weight of the rats for 5- and 6-week periods. It also includes the efficiency of each of the two diets mentioned above in terms of grams of diet consumed per gram of weight gained.

Even though the differences in growth in this experiment were not as wide as those obtained in 1945, fraction II again showed a striking superiority over fraction I, and over the butterfat and corn oil. Furthermore, the efficiency of the diet containing this fraction (II) was found higher than that containing whole butterfat. On comparing the gains on the butterfat itself to those on the corn oil, some superiority of the former is apparent with these sucrose diets.

TABLE 2
Growth gains and efficiency values on 28 per cent fat diets

	Butterfat	Corn Oil	Fraction I	Fraction II
	<i>g.</i>	<i>g.</i>	<i>g.</i>	<i>g.</i>
5-week gain	170	165	149	186
6-week gain	190	184	168	217
Grams diet/gram gain during 5th week	2.5	2.22

In repeating this experiment for further verification, it was decided to replace the corn oil diet by another containing Crisco (a partially hydrogenated cottonseed oil) as the source of fat. This was done in view of the reports by Kentie (7) and Boer *et al.* (2), (3), who claimed that the superiority of summer butter is due to its content of vaccenic acid and also because it was possible by Bertram's method (1) to isolate from Crisco a substance presumably corresponding to vaccenic acid. Also, the entire series was repeated on diets containing 10 per cent fat (table 1) in order to investigate the possibility that while a growth-promoting factor is being concentrated in fraction II, a retarding growth factor might be left in fraction I, and when fed at a 28 per cent level, would inhibit growth. Table 3 represents the various diets used and the corresponding weight gains during a 5-week period. During the sixth week, some of the rats were killed for microbiological analysis of their cecal flora.

TABLE 3
Growth gains during the 5-week period on 28 and 10 per cent fat diets

	Butterfat	Crisco	Fraction I	Fraction II
28% fat	143	144	157
10% fat	146	137	130	161

In comparing the values of the first experiment in table 2 to those displayed in table 3, generally poorer growth of the latter series becomes obvious. This is an unfortunate phenomenon in this type of study, and one which emphasizes the need for large numbers of experimental animals. However, within this series, the superiority of fraction II over the butterfat itself again is indicated both on the 28 and the 10 per cent level. The Crisco, from which have been isolated large amounts of the substance presumably corresponding to vaccenic acid, did not stimulate better growth than did butterfat.

In this experiment no significant difference seemed to have resulted from the varying levels of fat in the diets. However, work in progress points to the probability of the presence in certain fats of an inhibiting factor which exerts its effect to a noticeable degree when the fat represents a higher portion of the diet.

SUMMARY

September butterfat (1947) again was fractionated by crystallization from acetone at -5°C ., yielding a liquid fraction (II) which allowed rats to grow at a superior rate as compared with butterfat or corn oil, and a solid fraction (I) which gave a significantly slower growth rate.

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SACRAL DEFORMITY IN THE "WRYTAIL" ABNORMALITY IN CATTLE¹

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The importance and the possible mode of inheritance of "wrytail" in cattle have been presented by Atkeson and Warren (2) and by Atkeson *et al.* (1). These workers point out that the "wrytail" character has been observed in the Guernsey, Holstein-Friesian, Ayrshire, Brown Swiss and Jersey breeds. Their data indicate its inheritance as a single autosomal recessive. Observations of the present authors have extended to four breeds heretofore unreported and include 137 cattle of different ages and both sexes, as follows: Beef Shorthorns, 51; Aberdeen Angus, 39; Hereford, 17; Red Polled, 30. The only case of wrytail found was in one Red Polled cow in which the tailhead was set to the left.

A "wrytail", according to Atkeson *et al.* (1) is a malformation consisting of a distortion of the tail head, the base of the tail being set at an angle to the back bone instead of in line with it. The "tailhead" is the term commonly used by dairy cattle workers and judges to designate the area limited by the first three coccygeal vertebrae. The "wrytail" malformation therefore, would seem to be at or near the junction of the fifth sacral and first coccygeal vertebrae.

Roemmele (5) described a condition in Brown Allgauer cattle as being somewhat similar to "wrytail". Essentially, this malformation involved a twisting of the coccygeal vertebrae at a point posterior to the tail head. While the malformation described by Roemmele is similar in the effect on the vertebrae and intervertebral discs, the region affected makes it more nearly resemble "screwtail" (Knapp *et al.* (4)) than "wrytail".

EXPERIMENTAL

The present investigation involves a study of the anatomical features of the "wrytail" condition in a 7-year-old purebred Jersey female. Inasmuch as this was a case of definite wrytail and no similar analysis is known to have been attempted, it is thought the results may be of interest to others. In this case the tail was set to the right as indicated in figure 1. This condition was first observed when she was a 2-year-old. It is not

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known how long the character was present or if it was present at birth, as prior observations with this in mind had not been made. Three months after this picture was taken, the cow was slaughtered and a section of the rump, approximating 60 lb., was removed. This portion included the sacrum, most of the tail, and parts of the ilia. From the tissues thus involved, that portion of the tail and sacrum shown in figure 2 was prepared by removing the soft tissue, first by boiling water and, subsequently, by removing soft tissue with a scalpel and, finally, with steel wool. The sacrum was

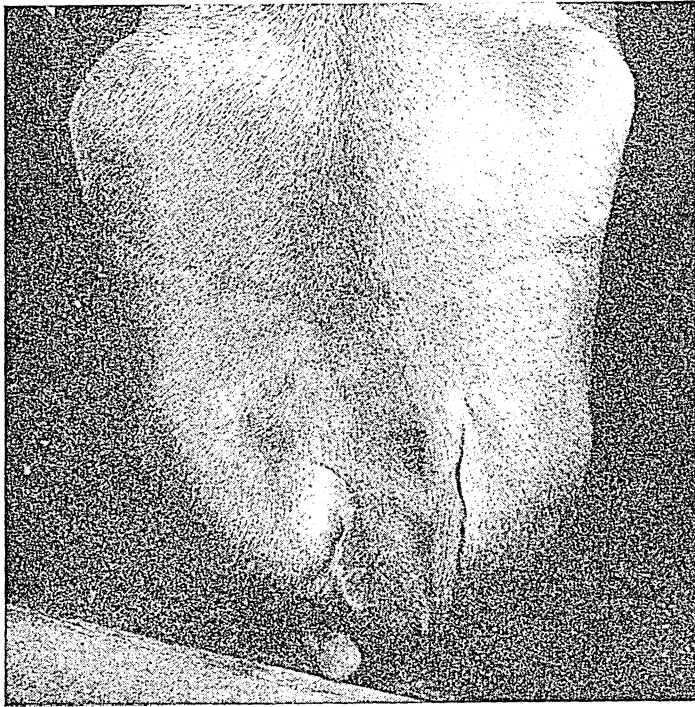


FIG. 1. Dorsal aspect of sacro-coccygeal region on a 7-year-old multiparous Jersey cow, no. 264.

then compared with the normal as described by Ellenberger and Baum (3) and by Sisson and Grossman (6). No evidence of trauma or inflammatory change (in terms of callus formation, thickening, or exostosis) was discovered.

The sacrum in this subject was 25 cm. in over-all length, 19 cm. in width anteriorly (alae) and 7.8 cm. in width at the extreme posterior extremity. Sacral segments I-IV, inclusive, were fused in their bodies, and in their spinous, transverse, and articular processes. The over-all length represented by fused segments I-IV, inclusive, was 20 cm. The fifth sacral segment was not fused at the junction of its body or transverse,

spinous, or articular processes with those of the fourth segment. The fifth dorsal and ventral sacral foramina thus were incomplete and that part of the lateral sacral crest, contributed to by the articular processes of sacral segments IV and V, also was incomplete. A lateral declination of approximately 12° to the right from the longitudinal axis common to sacral segments I-III, inclusive, to the axis common to sacral segments IV and V was noted. No declination was noted between the longitudinal axis of

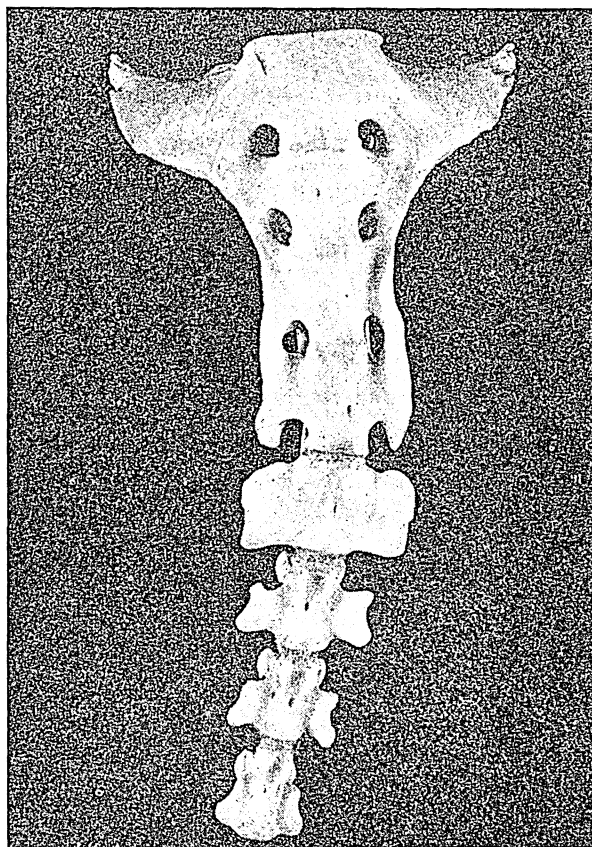


FIG. 2. Ventral aspect of sacrum and first three coccygeal vertebrae of Jersey cow no. 264, showing sacral deformity.

sacral segment V and that possessed in common by the first three coccygeal vertebrae (fig. 3).

Three radiographs were taken of this area, one before death and two after death. The one taken during life (11-15-46) demonstrated that the deformity did not lie in the anterior coccygeal region. The second (fig 3), taken of the frozen rump, revealed the site of the deformity to be in the sacrum. The final radiograph (fig. 4) was made of the sacro-coccygeal re-

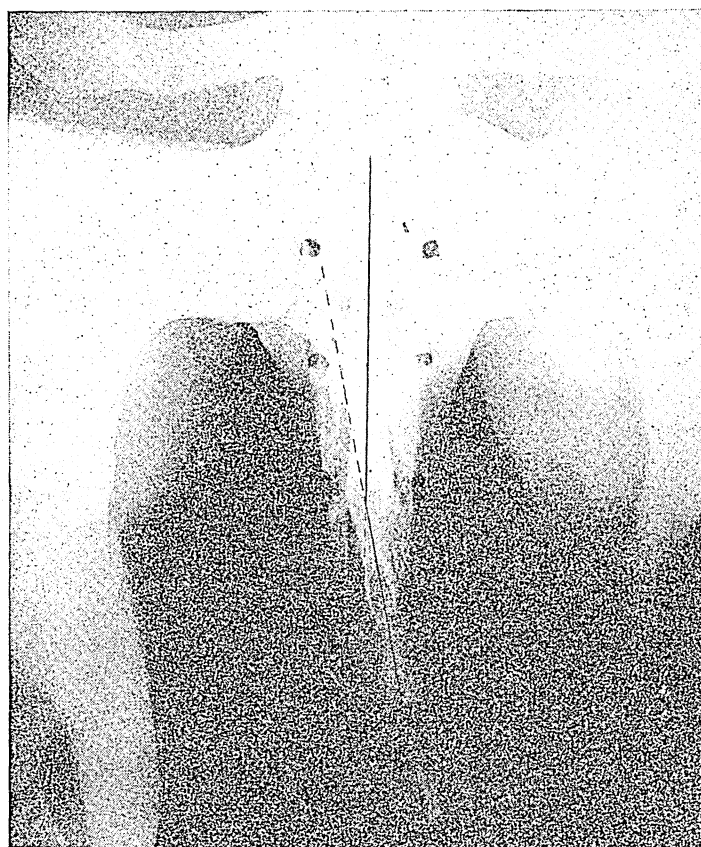


FIG. 3. Radiograph of sacro-coccygeal region, in section of rump removed from carcass at slaughter and then frozen. Dorsal view. Cow no. 264.

gion after removing the soft tissue. The specimen was laid flat on the cassette (*i.e.*, with the sacral alae depending over the edge) and a series of exposure made of the area apparently involved in the deformity (segments III-V, inclusive). The exposures were made on six portions of one large

TABLE 1
Detailed radiographic study of sacral segments III-V, inclusive

Exposure no.	Voltage	Distance	Tube current reading	Time
	(kilovolts)	(in.)	(milliamps.)	(sec.)
1	60	30	52	0.1
2	60	30	52	0.25
3	60	30	52	0.5
4	40	30	55	0.25
5	40	30	55	0.5
6	40	30	55	0.75

film, using lead plates to delineate the areas. Time and intensity variations were introduced, as noted in table 1.

The first series (exposures 1-3, inclusive) showed greater delineation of articulation or fusion (as the case might be) of adjacent segments, and midline detail in general. The second series (exposures 4-6, inclusive) showed greater delineation of transverse process structure.

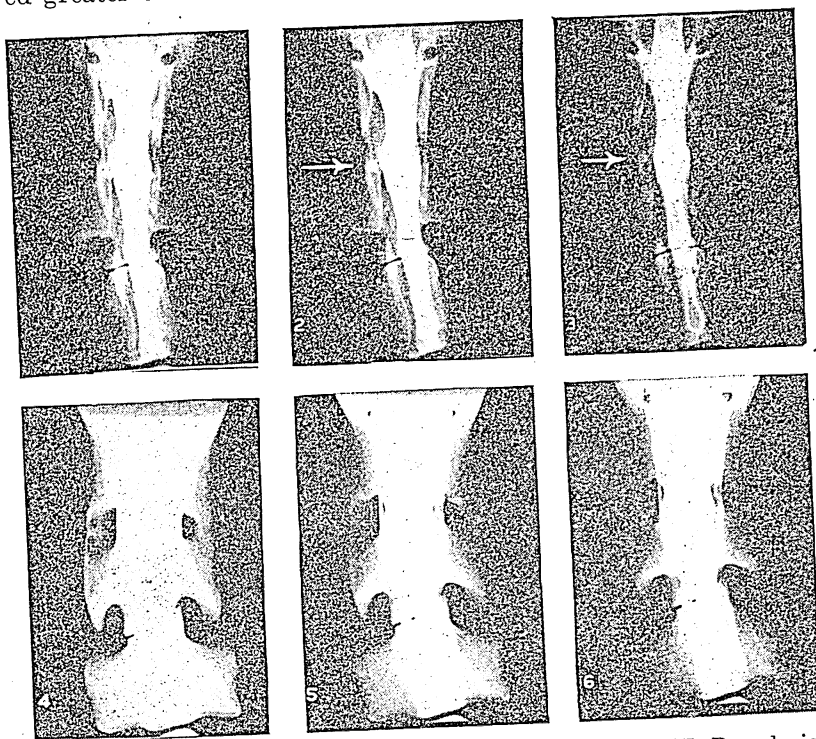


FIG. 4. Detailed radiographic study of sacral segments III-IV, Dorsal view (see table 1). Arrow indicates wedge-shaped area of fusion between segments III-IV. Cow no. 264.

An over-all lateral declination (right) of about 12° was noted, occurring at the fusion point of segments III-IV (7°) and within segment IV itself (5°). Since the specimen lay flat on the cassette at the time of exposure, and since the anode-film distance was relatively great and the perpendicularity of the central ray to the film was carefully checked, it was felt that linear and angular measurements made from the film would be valid. A measurement of declination of involved segments, made from the film (exposure 3, fig. 4), showed approximately 7° lateral declination (right) of the longitudinal axis (perpendicular to the line of fusion) of the anterior portion of sacral segment IV, from the longitudinal axis of segment III. An additional lateral declination of approximately 5°

(right) of the longitudinal axis of the posterior portion of segment IV (also perpendicular to the transverse axis of the articulation concerned, i.e., IV-V) from the longitudinal axis of the anterior portion, was noted.

On exposure 3, figure 4, a measurement of the intersegmental distance at the fusion point demonstrated a slight wedge-shape of that area. At its central and right portions, this area measured approximately 2 mm. in antero-posterior thickness. At its visibly expanded left extremity, it measured 3 mm. in antero-posterior thickness. The right lateral portion was not as amenable to accurate measurement on the X-ray film as the other portions of the fusion area, due to the partial obscurity cast by the spinous process. However, the wedge-shape and general measurements of this area were verified on the specimen itself. External measurements on the specimen are given in table 2.

Measurements also were made on right and left sagittal longitudinal axes of segment IV, at a distance of 12 mm. to either side of the ventral

TABLE 2
External measurements of intersegmental fusion areas of sacrum

Articulation (fusion area) of sacral segments	Transverse diameter of articulation ^a	Ventral intersegmental distance, antero-posterior		
		Left, 12 mm. from midpoint	Mid- point	Right, 12 mm. from midpoint
	(mm.)	(mm.)	(mm.)	(mm.)
I-II	39	5.0	2.0	5.0
II-III	35	3.5	2.75	3.25
III-IV	28	3.0	2.5	2.0

^a Calipers and dividers were used in making measurements.

midline (the distance 12 mm. was chosen arbitrarily, since differences seemed fairly pronounced and more easily measurable at that distance). The length of the right sagittal axis was found to be 47 mm. as compared to 50 mm. for the length of the corresponding left sagittal axis.

CONCLUSIONS

Examination of the sacral and coccygeal vertebrae of one animal showed the "wrytail" malformation to involve the sacrum rather than the tail-head in the case studied.

The extent and direction of the malformation as measured by the angle of declination was 12° right.

From radiographic and other observations, it appears that one locus of bone growth disparity lay in the fusion area between sacral segments III and IV. An additional declination (42 per cent of the total) occurred within the body of sacral segment IV.

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FROZEN HOMOGENIZED MILK. IV. KEEPING QUALITY OF FROZEN HOMOGENIZED MILK AFTER THAWING

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Previous studies (2, 5, 6, 7, 9) have shown that freezing and storage temperatures affect the physical character of homogenized milk. Later studies (3, 4, 8) showed that when homogenized milk was frozen, the solid components tended to concentrate in the lower portion of the sample. However, changes in the temperature at which frozen homogenized milk was stored did not affect materially the chemical composition of the different sections of quart samples. The concentration of the milk solids in the lower portion of the sample took place during the freezing process and apparently there was no further movement of these solids after the milk was frozen.

The literature does not contain information regarding the keeping quality of homogenized milk after it has been frozen and then thawed. The present study therefore was undertaken to determine the effect that storage of frozen homogenized milk has on its keeping quality after thawing.

PROCEDURE

Homogenized milk samples with a fat content of 3.8 per cent packaged in one-half pint paper containers by a commercial dairy in Washington, D. C., were used. This milk had been pasteurized at 155° F. for 30 minutes.

Samples of the fresh homogenized milk immediately were stored at 30.5° C., 15.5° C., 12.8° C., 7.22° C., and 1.67° C. Other samples were frozen immediately and held at -27.5° C. for various periods, after which they were thawed and then stored at the same temperatures as those at which the fresh milk samples had been held. At regular intervals a one-half pint sample of milk was removed from storage in order to determine its bacterial content, titratable acidity, pH, and flavor. The first three of these determinations were made in accordance with the methods outlined in Standard Methods for the Examination of Dairy Products (1). The plates were incubated at 37° C. for 48 hours. Flavor determinations were made by a panel of three men experienced in milk judging.

RESULTS

The flavor developments in homogenized milk held for different periods of time before and after freezing are shown in table 1. This table shows

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TABLE 1
Flavor of homogenized milk before and after freezing

Storage temperature	Storage period	Before freezing	Flavor quality									
			No. of days frozen milk stored at -27.5° C.									
(° C.)	(days)		3	17	24	31	45	66	87	111	129	150
Fresh 30.5	1	Normal
		Sour
15.5	1	Normal	Normal
	2	Normal	Normal
	3	Normal	Normal
	4	High acid.	Sl. oxidized
Fresh 12.8	Normal	Normal
	1	Normal	Normal
	2	Normal	Normal
	4	Normal	Normal
	5	Normal	Normal
	6	Normal	Normal
	7	Normal	Sl. old
	8	Sl. old	Sl. old
	8	Sl. old	Old
7.22	2	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
	4	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
	6	Normal	Normal	Sl. old	Sl. old	Sl. old	Sl. old	Sl. old	Sl. old	Sl. old	Sl. old	Sl. old
	8	Sl. old	Normal	Sl. old	Sl. old	Sl. old	Sl. old	Sl. old	Sl. old	Sl. old	Sl. old	Sl. old
	10	Sour	Old
	12	Sour	Sour	High acid	Sour	Old	Sour
1.67	2	Normal	Normal	Normal	Normal	Normal
	4	Normal	Normal	Normal	Normal	Normal	Normal
	7	Normal	Normal	Normal	Normal	Normal	Normal
	9	Normal	Normal	Normal	Normal	Normal	Normal
	11	Sl. old	Normal	Sl. old	Sl. old	Sl. old
	14	Sl. old	Sl. old	Sl. old	Sl. old	Sl. old	Sl. old
	16	Old	Sl. old	Sl. old	Sl. old	Sl. old	Sl. old
	18	Old-unclean	Old	Old	Old	Old	Old
	21	Bitter	Unclean

TABLE 2
Bacterial growth in homogenized milk before and after freezing

Storage temperature	Storage period	Before freezing	Bacteria per millimeter											
			No. of days frozen milk stored at -27.5° C.											
(°C.)	(days)		3	17	24	31	45	66	87	111	129	150		
Fresh		800												
30.5	1	11,600,000						11,248,000	31,400,000	70,000,000	30,000,000			
15.5	1	850	600											
	2	6,500	1,300											
	3	278,000	133,000											
	4	4,000,000	1,980,000											
Fresh		300												
12.8	1	400												
	2	400	500											
	4	800	300											
	5	5,300	300											
	6		4,800											
	7	975,000	80,000											
	8	560,000	351,000											
7.22	2	490	1,300	400	900									
	4	7,000	600	600	300	400								
	6	152,000	123,000		117,000	167,000								
	8	2,770,000	160,000	69,000	780,000	140,000								
	10	6,000,000	220,000	4,860,000	11,000,000	165,000								
	12	12,000,000	3,500,000	21,500,000	92,000,000	310,000	1,230,000							
1.67	2	800						300	1,000		1,600			
	4	1,000						300	200	300	1,000	1,100		
	7	1,200						300	500	500	600	1,900		
	9	1,600						300	400	1,100	600	7,400		
	11	5,000						11,200	12,000	123,000		36,000		
	14	180,000						78,000	11,400	95,800	600,000	13,800,000		
	16							160,000			1,000,000			
	18	960,000						420,000	342,000					
	21	1,500,000						1,000,000	780,000					

that storage has practically the same effect on the flavor of both the fresh homogenized milk and the homogenized milk that had been held in the frozen state. The samples that were stored at 30.5° C. were sour when examined at the end of 24 hours. Those which previously had been stored for 111 days and 129 days at -27.5° C. were unclean or putrid, indicating that the rate of flavor deterioration may be slightly more rapid in milk that has

TABLE 3
Titrateable acidity development in homogenized milk before and after freezing
(Standard methods (1) procedure used)

Storage tempera- ture	Storage period	Titrateable acidity (% lactic acid)											
		Before freezing	No. of days frozen milk stored at -27.5° C.										
			3	17	24	31	45	66	87	111	129	150	
(°C.)	(days)												
Fresh	0.12
30.5	1	0.36	0.37	0.36	0.34	0.35
15.5	1	0.12	0.12
	2	0.12	0.12
	3	0.12	0.12
	4	0.17	0.16
Fresh	0.12
12.8	1	0.12	0.12
	2	0.12	0.12
	4	0.12	0.13
	5	0.12	0.13
	6	0.13
	7	0.13	0.13
	8	0.14	0.12
7.22	2	0.12	0.12	0.12	0.12
	4	0.12	0.12	0.12	0.12	0.12	0.12
	6	0.13	0.13	0.13	0.12	0.13
	8	0.14	0.13	0.13	0.14	0.13	0.15
	10	0.28	0.13	0.17	0.16	0.15	0.16
	12	0.45	0.27	0.20	0.33	0.16
1.67	2	0.12	0.13	0.13	0.13	0.14
	4	0.12	0.12	0.13	0.12	0.15	0.14
	7	0.13	0.12	0.12	0.14
	9	0.13	0.14	0.12	0.12	0.15	0.14
	11	0.14	0.13	0.12	0.13	0.15
	14	0.15	0.13	0.12	0.17	0.14	0.14
	16	0.15	0.13
	18	0.18	0.18	0.15
	21	0.20	0.16

been held in the frozen state. However, there appeared to be no appreciable difference in the rate of flavor deterioration between the two milks when lower storage temperatures were used.

Table 1 further shows that frozen homogenized milk of good quality can be stored at usual storage temperatures after thawing without deterioration in flavor for longer periods of time than usually are required to hold fluid milk before use.

To determine whether bacteria multiply faster and acidity develops more rapidly in homogenized milk which has been frozen and then thawed than in the corresponding fresh homogenized milk, samples of the two milks were stored at different temperatures. The initial bacterial count, acidity, and pH values were determined prior to placing the milks in storage. After different storage periods, these determinations were repeated. The results are given in tables 2, 3 and 4.

TABLE 4
pH changes in homogenized milk before and after freezing

Storage tempera- ture	Storage period	pH										
		Before freezing	No. of days frozen milk stored at -27.5° C.									
			3	17	24	31	45	66	87	111	129	150
(°C.)	(days)											
Fresh	6.73
30.5	1	5.40	5.28	5.43	5.96	5.46
15.5	1	6.65	6.52
	2	6.61	6.52
	3	6.52	6.57
	4	6.37	6.43
Fresh	6.70
12.8	1	6.69	6.18
	2	6.61	6.65
	4	6.33	6.51
	5	6.70	6.43
	6	6.46
	7	6.60	6.45
	8	6.44	6.47
7.22	2	6.63	6.52	6.55	6.46
	4	6.52	6.57	6.50	6.37	6.64	6.50
	6	6.58	6.55	6.42	6.43	6.42
	8	6.50	6.46	6.50	6.52	6.52	6.48
	10	6.01	6.52	6.23	6.48	6.48	6.48
	12	5.34	5.84	6.00	5.82	6.48
1.67	2	6.50	6.57	6.61	6.62	6.74
	4	6.58	6.58	6.73	6.64	6.80	6.68
	7	6.66	6.62	6.62	6.48
	9	6.50	6.58	6.68	6.52	6.68	6.50
	11	6.48	6.56	6.70	6.60	6.62
	14	6.50	6.58	6.72	6.54	6.62	6.69
	16	6.52	6.62
	18	6.40	6.49	6.52
	21	5.90	6.48

Table 2 shows that, as previously reported (3), freezing milk and storing it in the frozen state had a tendency to lower the number of bacteria per ml. as determined by the standard plate count. The initial bacterial counts of the fresh homogenized milk were higher than those of the corresponding homogenized milk which had been held in the frozen state. These differences, however, were not consistently reflected in the counts made after various periods of storage. This indicates that from a bacteriological standpoint there is no significant difference between fresh homogenized milk and homogenized milk which has been held in the frozen state.

Tables 3 and 4 show that acidity develops at practically the same rate in homogenized milk when thawed, after being held in the frozen state, as it does in fresh homogenized milk when both are held under similar conditions.

CONCLUSIONS

The changes in flavor during the storage of homogenized milk which has been held in the frozen state and then thawed were similar to those of fresh homogenized milk. Frozen homogenized milk of good quality may be stored at usual storage temperatures after thawing without deterioration in flavor for longer periods than usually are required to hold fluid milk before use.

From a bacteriological standpoint, as determined by standard plate counts, there was no significant difference between homogenized milk which has been stored in the frozen state and then thawed and fresh homogenized milk.

Homogenized milk that had been stored in the frozen state and then thawed showed no significant difference from fresh homogenized milk in the development of acid as measured by titratable acidity and pH determinations.

The authors wish to express their appreciation to Edith Giltner and Elmina Dickson for their assistance with the analytical determinations.

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FROZEN HOMOGENIZED MILK. V. EFFECT OF AGE BEFORE FREEZING ON THE KEEPING QUALITY OF FROZEN HOMOGENIZED MILK

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Earlier studies (2, 6, 7, 9, 10) have shown that freezing and storage temperatures affect the physical character of homogenized milk. Previous studies (3, 4, 8) also have shown that when homogenized milk was frozen, the solid components tended to concentrate in the lower portion of the sample during the freezing process; apparently there was no further movement of these solids after the milk was frozen. A more recent study (5) has shown that frozen homogenized milk of good quality can be stored at usual storage temperatures after thawing without deterioration for longer periods of time than usually are necessary before use.

The literature does not contain information regarding the effect of the age of homogenized milk before freezing on its keeping quality after freezing. The present study was undertaken to determine the effect of age before freezing on the keeping quality of frozen homogenized milk.

PROCEDURE

Homogenized milk samples with a fat content of 3.8 per cent packaged in one-half pint paper containers by a commercial dairy in Washington, D. C., were used. The milk had been pasteurized at 155° F. for 30 minutes. Fifty-six samples were taken directly from the filler and divided into seven groups of eight samples each. One sample was examined immediately for flavor, bacterial count, coliform organisms, titratable acidity, pH, and sediment and the remaining seven samples of this group were placed in a freezer held at -17.5° C.² The remaining samples were held at 1.67° C. Eight samples, each representing a separate group, were removed from the 1.67° C. storage after 12, 24, 48, 72, 96 and 120 hours. Each time, one of the eight samples of the respective group was used for laboratory examination and the remaining seven were placed in a freezer at -17.5° C. The samples were removed from the -17.5° C. storage and thawed for laboratory examination after having been held in the frozen state for 5, 28, 38, 47, 56, 76, and 86 days. The determinations for bacterial content,

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² The thermostat controlling the temperature of this freezer had sufficient lag to cause a temperature variation of about eight degrees.

TABLE 1 (continued)
Effect of age before freezing on the keeping quality of frozen homogenized milk

	Sediment									
	(ml.)	(ml.)	(ml.)	(ml.)	(ml.)	(ml.)	(ml.)	(ml.)	(ml.)	(ml.)
0	0.02	0.02	0.25	0.40	0.60	1.20	1.20	1.20	1.20	4.50
12	0.03	0.02	0.10	0.60	0.25	0.70	1.30	1.30	1.30	3.50
24	0.03	0.02	0.10	0.40	0.30	1.10	2.20	2.20	2.20	3.80
48	0.02	0.02	0.55	0.20	0.50	0.60	2.70	2.70	2.70	2.50
72	0.02	0.03	0.30	0.90	0.60	1.10	2.60	2.60	2.60	4.50
96	0.02	0.02	0.40	0.50	1.30	1.60	1.60	1.60	2.20
120	0.02	0.02	0.20	0.40	0.65	0.60	2.00	2.00	2.00	3.00

	Acidity									
	Titra- table	pH	Titra- table	pH	Titra- table	pH	Titra- table	pH	Titra- table	pH
0	0.115	6.67	0.125	6.62	0.115	6.62	0.115	6.68	0.130	6.56
12	0.125	6.79	0.120	6.63	0.115	6.58	0.110	6.62	0.130	6.55
24	0.120	6.64	0.120	6.61	0.120	6.60	0.110	6.63	0.125	6.61
48	0.120	6.60	0.115	6.53	0.115	6.72	0.115	6.67	0.125	6.59
72	0.120	6.63	0.110	6.59	0.110	6.72	0.115	6.61	0.130	6.63
96	0.120	6.65	0.115	6.65	0.115	6.58-	0.115	6.60	0.130	6.62
120	0.120	6.64	0.115	6.62	0.110	6.62	0.115	6.63	0.130	6.42

^a ox. = oxidised

^b + and - denote presence or absence of coliform organisms in 1-ml. samples.

coliform organisms, titratable acidity, and pH were made in accordance with the methods outlined in Standard Methods for the Examination of Dairy Products (1). The plates were incubated at 37° C. for 48 hours. Flavor determinations were made by a panel of three men, all of whom were experienced in milk judging. The sediment was determined by the method used by the authors in their earlier studies (2, 3).

RESULTS

The effect of age before freezing on the keeping quality of frozen homogenized milk is shown in table 1. The milk was of good flavor throughout the 120 hours that it was held at 1.67° C. before freezing. The flavor of the milk remained good when it was held in the frozen state for 47 days, regardless of its age before freezing. Some of the samples that were thawed after they had been frozen for 56 days and for 76 days had developed a slight oxidized flavor. However, there was no correlation between the age of the samples before freezing and the development of this flavor. When the samples were thawed after holding in the frozen state for 86 days, they had a stale and oxidized flavor. There was an insignificant tendency for these flavors to be more pronounced as the age of the samples before freezing was increased.

Table 1 also shows that there was no significant change in the bacterial content of the samples either in the 120 hours that they were held at 1.67° C. or in the 89 days that they were held in the frozen state at -17.5° C. There apparently was a slight coliform contamination of the milk that was used in the preparation of the samples. With the exception of those samples which were held at 1.67° C. for 24 hours before freezing, at least one sample in each age group was positive for coliform organisms in 1-ml. portions either before or after freezing. Two of the samples before freezing and two each of those held in the frozen state for 5, 28, 47 and 56 days gave positive coliform tests. Of these samples held in the frozen state for 47 and for 86 days, one each gave a positive test. There was no correlation between the positive coliform tests in the samples before and after freezing.

Table 1 shows further that the quantity of sediment remained low and constant in the milk samples before they were frozen. It did not increase when the samples were held in the frozen state for 5 days. When held for 38 days, however, the quantity of sediment had materially increased and it continued gradually to increase as the storage time lengthened. Significant separation, as shown by the sediment readings, had occurred when the samples were thawed after they had been held for 47 days in the frozen state, and considerable sediment was present in all the samples when they were thawed after having been frozen for 89 days. There was, how-

ever, no correlation between the degree of separation and the age of the sample prior to freezing.

The acidity as shown by titration and by pH determinations did not vary significantly either before the samples were frozen or while they were held in the frozen state. The titratable acidity ranged from 0.110 to 0.130 per cent and the pH values from 6.51 to 6.79. These variations were well within the limits of experimental error. The titratable acidity values were lower than usually encountered because of the dilution technic employed.

CONCLUSION

It is recognized that homogenized milk should be frozen as soon as possible after processing, but if the milk is of good quality it may be kept as long as 120 hours at 1.67° C. before freezing without adversely affecting the keeping quality of the frozen product.

The authors wish to express their appreciation to Edith Giltner and Elmina Dickson for their assistance with the analytical determinations.

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FERTILITY LEVEL OF BULL SEMEN DILUTED AT 1:400 WITH AND WITHOUT SULFANILAMIDE

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The basic advantage of artificial insemination, the ability to breed more cows to superior sires than can be done in natural service, can be realized on a broad practical basis only if the single ejaculate of a superior bull can be divided and many cows bred with it. The practical difficulty involved in inseminating extremely small volumes of semen has resolved the problem to standardizing the volume inseminated at 1.0 ml. and diluting the semen with appropriate amounts of diluter. This was done to reduce the spermatozoa numbers to a level consistent with maximum efficiency of use of semen and optimum fertility.

Earlier studies (3, 4, 5) have shown that bull semen may be diluted at levels as high as 1 part of semen to 100 parts of the yolk-citrate diluter with no detectable effect on the fertility level. The present report deals with experiments designed to test even wider dilution rates.

EXPERIMENTAL PROCEDURE

The investigations reported here were conducted in cooperation with the New York Artificial Breeders' Cooperative, Inc. The general methods used in handling the semen and the methods used in determining the results of each insemination have been reported earlier (3, 4, 5). Two investigations were made. The first was to study the effect of levels of dilution above 1:100 when the yolk-citrate diluter was used. This diluter was composed of equal parts fresh egg yolk and a buffer composed of 3.6 g. of sodium citrate dihydrate per 100 ml. of water distilled in glass and autoclaved for 20 minutes at 15 lb. pressure.

Preliminary evidence suggested that some decrease in fertility might be expected at dilution rates above 1:100. Therefore, a carefully designed experiment was conducted using the semen of one bull of consistently high fertility. The experiment was in the form of a 4 × 4 Latin square, each of four ejaculates being split into four aliquots and each of these aliquots being diluted at rates of one part of semen to 100, 200, 400 and 800 parts of the yolk-citrate diluter. It was impossible for each of the 75 different inseminators involved in this study to use semen diluted at each level for each of the ejaculates used. Therefore, the inseminators were divided arbitrarily into four groups. Each group received semen diluted at one

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single rate for one particular collection, but each group received all dilution rates at some time during the experiment. Such a design, although somewhat cumbersome to manipulate in the central headquarters from which the semen was shipped, presented no difficulty in the field and permitted precise measurements of the results.

The second experiment was with 19 different bulls and was designed as a randomized block experiment. Each bull was considered as a block. Five collections were made from each bull with intervals of approximately 10 days between collections. Dilution rates were assigned at random to these collections. Each semen collection was diluted at one rate, i.e., one part of semen to 100, 150, 200, 300 or 400 parts of the diluter used. The semen was shipped to all inseminators using semen of that particular breed. Four breeds were represented. The diluter used was composed of equal parts egg yolk and a sodium citrate-sulfanilamide buffer consisting of 3.6 g. sodium citrate dihydrate and 0.6 g. sulfanilamide made up to 100 ml. with water distilled in glass. The final concentration of sulfanilamide in the diluter was 300 mg. per 100 ml. This experiment, although not giving as precise estimates of the sources of random variation as the first, was much more extensive in scope, and more general conclusions and recommendations for practice could be drawn from it.

A statistical study also was made of the relation between the number of spermatozoa inseminated and fertility level in the case of approximately 700 ejaculates of semen routinely used in artificial insemination between January 1 and August 31, 1947.

RESULTS

In the first experiment the average numbers of spermatozoa in 1 ml. of diluted semen at each dilution rate were: for 1:100, 14,580,000; for 1:200, 7,330,000; for 1:400, 3,670,000 and for the 1:800 dilution rate, 1,840,000 spermatozoa per 1 ml. inseminated. The results are presented in table 1 and include services to cows bred for the first and for the second time in a service period. An analysis of covariance using services as the independent and 165-day (5-month) non-returns as the dependent variable showed that the differences observed were significant, the 1:400 and 1:800 rates being different from the 1:100 rate. Also, it should be noted that the cows bred with the semen diluted at 1:100 apparently held to service better than did the cows bred with semen diluted at the higher rates. This fact was of considerable interest and prompted the second experiment.

In the second experiment the average number of spermatozoa in 1 ml. of the diluted semen was: for 1:100, 12,060,000; 1:150, 8,490,000; 1:200, 6,340,000; 1:300, 4,160,000 and for the 1:400 dilution rate, 3,290,000 spermatozoa. The means of the semen quality characteristics are presented in table 2. Statistical analyses of these data show that none of the differ-

TABLE 1

Fertility of the semen of one bull diluted at various levels with yolk-citrate, 45, 75 and 165 days after insemination

	Ratio of semen to diluter				Total
	1: 100	1: 200	1: 400	1: 800	
Services	98	106	82	70	356
% non-returns					
Av. 45 days	76.5	63.2	56.1	61.4	64.6
Av. 75 days	67.3	50.9	45.1	47.1	53.4
Av. 165 days	61.2	48.1	36.6	44.3	48.3

ences were significant. Thus, it is concluded that the design did not bias the experiment towards different qualities of semen for use in preparation of different dilutions.

The results of the inseminations are shown in table 3. They represent only those services to cows being bred for the first time in a service period and are given as 45-day (1 month), 75-day (2 month) and 165-day (5 month) non-returns to service. Statistical analysis of the data indicated that the mean differences in fertility level were not statistically significant. In fact, the variance due to treatments from the analysis of covariance and that due to random variation in the data were almost identical, the F' value being 1.01 for 75-day non-returns and 0.78 for 165-day non-returns. However, a trend toward a decrease in fertility with each decrease in the number of spermatozoa inseminated is apparent in the mean values for each dilution rate given in table 3. The correlation between the number of spermatozoa inseminated and the fertility of each ejaculate used in the experiment was 0.24, a small but significant figure at the 5 per cent level of probability. The relationship indicated by the correlation coefficient explained but a minor portion of the variance in fertility level observed. The regression of fertility level on spermatozoa numbers inseminated was linear. Between the limits of numbers of spermatozoa per insemination used, the calculated regression formula was $Y = 51.27 + 0.777X$, where Y = estimated per cent of 165-day non-returns and X = the number of spermatozoa inseminated. This

TABLE 2

Average of the semen quality characteristics for each dilution rate

	Ratio of semen to diluter				
	1: 100	1: 150	1: 200	1: 300	1: 400
Initial motility, %	72.1	71.8	71.3	71.8	71.1
Concentration, 1,000's/ mm. ³	1,218	1,282	1,274	1,253	1,318
Methylene blue reduction time, min.	5.0	4.9	4.7	4.9	4.6

result is equivalent to a change in fertility level of approximately 0.8 per cent for each change of one million spermatozoa between the limits used in the experiment.

Similar calculations for approximately 700 ejaculates used routinely in artificial insemination resulted in a smaller but a statistically significant regression coefficient. The regression equation was $Y = 56.86 + 0.3146X$ or equivalent to a change of approximately 0.3 per cent in fertility for each change of one million spermatozoa inseminated. However, the regression calculated on this latter data was for a different range of spermatozoa numbers inseminated than was the case of the experimental data. The extreme ranges of spermatozoa numbers inseminated in the routine work were from 6,700,000 to 34,600,000 per insemination, with the mean being 14,700,000 spermatozoa. In contrast, the range in the last experiment was from 2,360,000 to 15,300,000 spermatozoa per insemination.

TABLE 3

Fertility of the semen of 19 bulls diluted at various rates with yolk-citrate sulfanilamide, 45, 75 and 165 days after insemination

	Ratio of semen to diluter					Total
	1: 100	1: 150	1: 200	1: 300	1: 400	
Total services	1408	1580	1581	1379	1395	7343
% non-returns						
Av. 45 days	69.1	64.3	66.7	62.6	62.6	65.1
Av. 75 days	60.9	56.3	58.4	55.0	52.8	56.7
Av. 165 days	58.1	53.2	55.4	51.3	48.5	53.3

DISCUSSION

This series of investigations to determine the optimum dilution rate for fertile bull semen and the minimum number of bovine spermatozoa required for maintenance of optimum fertility in artificial insemination appears to present a number of problems, some of which have been answered only partially. In the first place, the data reported earlier (3, 4, 5) showed no decrease in fertility as the numbers of spermatozoa inseminated were decreased from approximately 400 million down to approximately 13 million spermatozoa per insemination. In those experiments, yolk-citrate diluter, made up of equal parts of fresh egg-yolk and a solution containing 3.6 g. or slightly more of sodium citrate dihydrate per 100 ml. of water distilled in glass was used.

When the numbers of spermatozoa were reduced further by increased dilution with the same diluter used in the first experiment reported here, the decrease in fertility noted was large and the differences were significant. This fact suggested that the minimum number of spermatozoa per insemination for optimum fertility had been reached. In contrast, however, the second experiment, in which sulfanilamide was added to the diluter, failed

to show the same great decrease in fertility level over a range of spermatozoa numbers similar to that used in the first experiment. A downward trend in fertility was suggested by the regression calculation. An even smaller downward trend in fertility with decreasing spermatozoa numbers was shown for semen used in routine artificial insemination work in which the yolk-citrate sulfanilamide diluter was regularly used.

These facts suggest that the curve of declining fertility with decreasing spermatozoa numbers probably is a logarithmic one in which the plateau of approach to the optimum is long and the slope very small. However, as the minimum is approached, the rate of decrease in fertility accelerates, the slope of the curve becomes greater and the fertility level probably reaches zero before spermatozoa numbers reach that level.

Secondly, the data presented here and other evidence from this laboratory suggest that the position of the curve, although perhaps not its slope, may be altered to some degree by the diluter in which the spermatozoa are suspended. It has been shown that the livability of bull spermatozoa is shortened by greater dilutions (4). Also, it has been shown that bull spermatozoa in low concentrations are harmed by oxygen (2). Sulfanilamide depresses the oxygen consumption by spermatozoa, stimulates increased livability (1), and improves the fertility of spermatozoa used for insemination after storage (6). It is not known that sulfanilamide will increase the inherent fertility of bull semen if that semen were used for insemination immediately after collection. Rather, the effect in increasing fertility earlier observed (6) is believed to be due to prolongation of innate fertilizing capacity rather than an actual increase in the potential.

Based on these observations it is suggested that the addition of sulfanilamide to the diluter in the last experiment reported here preserved the life of the spermatozoa in low concentrations better than was done by the yolk-citrate. Thus, the downward acceleration of the fertility level was partially prevented at the levels of spermatozoa numbers used. However, it is believed that with somewhat lower numbers of spermatozoa, the accelerated decrease in fertility would be observed. Until more fundamental information is available leading to control of the metabolic processes involved, it appears that the minimum number of spermatozoa from fertile bulls which should be used for insemination of cows rests at between 5 and 10 millions per insemination. In the case of particularly valuable proved sires that are highly fertile, lower numbers of spermatozoa can be used but a sacrifice in fertility level probably would result.

Finally, it should be emphasized that these experiments were carried out under field conditions in which shipment of semen was routine. Most inseminations were made the second, third and fourth days after the semen was collected. The semen was from normal bulls of high fertility. It was of excellent quality, as shown in table 2. However, these experiments do not

enable one to speculate on the probable fertility of highly diluted semen from bulls of low fertility. Nor do the minimum numbers mentioned above imply that bulls producing semen of low spermatozoa count will be fertile.

SUMMARY

In one investigation it was found that the practical limit of dilution rate was about 1:100 when the yolk-citrate diluter was used. In another experiment involving 7,343 inseminations when sulfanilamide was added to the yolk-citrate diluter at the rate of 300 mg. per 100 ml., no difference was found in fertility level between dilution rates of one part of semen to 100, 150, 200, 300 and 400 parts of the yolk-citrate-sulfanilamide diluter.

However, a trend downwards amounting to 0.8 per cent in fertility level for each decrease of 1 million spermatozoa inseminated was observed, over the range of 2.36 to 15.30 millions of spermatozoa inseminated. The probable reasons for the different results of the two experiments are discussed.

With present handling and insemination techniques, it is suggested that the minimum number of spermatozoa consistent with optimum fertility rests at 5 to 10 millions from bulls of known fertility.

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AN ANALYSIS OF THE RESULTS OF THE 1947 COLLEGIATE STUDENTS' INTERNATIONAL CONTEST IN JUDGING DAIRY PRODUCTS

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A few items on the score cards used in the Collegiate Students' International Contest in the Judging of Dairy Products frequently have been questioned as to their real values in testing the judging abilities of the contestants. The inference sometimes is made that certain items are scored as effectively by the lower ranking contestants as by the higher ranking contestants. If this is true, these items may have little weight in determining the judging abilities of contestants. Some coaches of dairy products judging teams have felt that high ranking contestants attain their standing, in part at least, because of extreme conservatism in certain phases of scoring.

The contestants' score cards from the 1947 contest were made available to determine if high standings were attained without actually showing proficiency in judging. Also, it seemed desirable to determine if any techniques were employed by the winning contestants that could be used in the training of judging teams.

PROCEDURE

The data included herein were obtained from a study of the score cards of 57 contestants. Each scored ten samples of creamery butter, milk, Cheddar cheese and vanilla ice cream. Hereafter, these products will be designated as butter, milk, cheese and ice cream. Two hundred and twenty-eight contestant cards were examined and 2,280 judgments were involved.

The contestants' score cards for butter, milk, cheese and ice cream were grouped into quartiles. The first quartile consisted of the cards of the 14 contestants scoring highest in the judging of that specific product; the second quartile contained the cards of the 14 next highest contestants, and so on. The quartiles for the different products may or may not have represented the same contestants. It should be pointed out that the fourth quartile included cards from contestants who through carelessness failed by omission or commission to score properly the various items and, therefore, were given the maximum penalty. For this reason the data obtained from the cards in the fourth quartile may not be significant.

DISCUSSION OF THE DATA

An examination of the data presented in tables 1 to 4, inclusive, reveals

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TABLE 1

The grades (points lost) of the three high individuals and quartiles of contestants in the judging of butter

Class	Points lost in scoring								Total
	Flavor		Body & texture		Color		Salt		
	Score	Criti- cism	Score	Criti- cism	Score	Criti- cism	Score	Criti- cism	
1st individual.....	7.00	1.00	0.0	0.0	0.0	0.0	0	0	8.00
2nd “ ”	7.00	2.50	0.0	0.0	0.0	0.0	0	0	9.50
3rd “ ”	9.00	2.00	0.0	0.0	0.0	0.0	0	0	11.00
Av. of 3 high individuals	7.66	1.83	0.0	0.0	0.0	0.0	0	0	9.49
1st quartile	9.04	3.25	0.29	0.43	0.0	0.0	0	0	13.01
2nd “ ”	11.39	3.93	0.96	1.43	0.0	0.0	0	0	17.71
3rd “ ”	12.00	3.71	2.14	1.29	0.11	0.07	0	0	19.32
4th “ ”	16.43	5.20	3.67	3.67	0.0	0.0	0	0	28.97

information very similar to that noted in previous studies (1, 2); *i.e.*, the high ranking contestants in each product attained that position because they knew how to score properly the "flavor" and "body and texture."

The following deductions from the data may be helpful:

Flavor. The three high contestants in the scoring of the specific products were not significantly better than the others in criticizing the flavor of cheese, ice cream and milk. However, the three high individuals showed superior ability to describe and evaluate the flavor of butter. Generally all contestants lost more points in placing a score on flavor than they did in recognizing a flavor.

Body and texture. In cheese judging, the two high individuals surpassed their close competitors both in recognizing defects of body and tex-

TABLE 2

The grades (points lost) of the three high individuals and quartiles in the judging of Cheddar cheese

Class	Points lost in scoring						Total
	Flavor		Body & texture		Color		
	Score	Criticism	Score	Criticism	Score	Criticism	
1st individual	9.00	6.00	4.50	1.50	1.00	2.00	24.00
2nd “	9.00	5.50	4.00	3.00	1.50	3.00	26.00
3rd “	10.50	6.50	7.00	3.50	1.00	2.00	30.50
Av. of 3 high individuals	9.50	6.00	5.16	2.66	1.16	2.33	26.81
1st quartile.....	11.18	6.14	7.39	4.04	1.14	2.14	32.03
2nd “	13.89	7.18	7.86	5.79	1.39	2.57	38.68
3rd “	15.04	7.00	11.64	5.96	1.18	2.36	43.18
4th “	20.10	7.57	12.50	6.60	2.10 ^a	3.10	51.97

^a Only 13 contestants involved. Data from two contestants were so abnormally out of line they were not included.

TABLE 3

The grades (points lost) of the three high individuals and quartiles in the judging of milk

Class	Points lost in scoring					Total
	Flavor		Sediment	Container & closure		
	Score	Criticism	Score	Score	Criticism	
1st individual.....	8.50	6.00	3.00	0.50	0.50	18.50
2nd “.....	18.00	4.00	2.25	0.25	1.00	25.50
3rd “.....	12.00	6.50	2.50	2.25	3.50	26.75
Av. of 3 high individuals.....	12.83	5.50	2.58	1.00	1.66	23.57
1st quartile.....	16.50	5.62	4.08	2.09	1.99	30.28
2nd “.....	20.96	6.51	4.68	1.48	2.42	36.05
3rd “.....	25.14	6.35	6.17	1.96	2.99	42.61
4th “.....	30.30	7.08	8.11	2.55	3.03	51.07

ture and in weighing the criticism. In body and texture of butter, the first three individuals agreed precisely with the judge and the contestants in the first quartile did not vary too widely from the judge. However, in criticizing ice cream, the three high contestants were not significantly better than those in the first quartile; in evaluating the criticism, the two highest individuals were somewhat superior to their immediate competitors.

Miscellaneous items. Neither the judges nor the contestants criticized the salt content in butter in this contest. However, some contestants criticized the color of butter, but the judge did not do so.

In the judging of the color of cheese, the three high individuals did no better than did those in the first three quartiles.

Practically all contestants scored and criticized similarly the melting quality of ice cream. However, the scoring of color of ice cream was evidently a "booby trap" for some of the contestants, especially those in the fourth quartile.

TABLE 4

The grades (points lost) of the three high individuals and quartiles in the judging of vanilla ice cream

Class	Points lost in scoring								Final grade
	Flavor		Body & texture		Melting quality		Color		
	Score	Criticism	Score	Criticism	Score	Criticism	Score	Criticism	
1st individual.....	12.00	5.70	3.50	2.50	2.50	3.00	0.0	0.0	29.20
2nd “	15.50	6.50	4.50	2.50	1.50	1.00	0.0	0.0	31.50
3rd “	16.00	7.50	6.00	2.00	0.50	0.00	0.0	0.0	32.00
Av. of 3 high individuals	14.50	6.57	4.66	2.33	1.50	1.33	0.0	0.0	30.89
1st quartile.....	16.17	6.05	5.79	2.79	1.93	2.04	0.04	0.07	34.88
2nd “	17.39	6.34	7.93	4.76	2.14	2.64	0.32	0.14	41.66
3rd “	22.04	7.32	9.38	5.26	2.29	2.36	0.29	0.29	49.23
4th “	21.57	7.52	10.50	7.33	2.23	2.30	4.30	0.33	56.08

In scoring sediment of milk, it is possible that the three high individuals retained a more accurate mental picture of the sediment standards than other contestants and thereby attained a better score. The item "container and closure" of milk evidently had been so well impressed upon the minds of all contestants that little variation existed in the scoring and criticizing of this item.

The fourth quartile. Table 5 was compiled to demonstrate in what area the contestants of the fourth quartile failed most seriously. The data were secured by subtracting the average grade of the first quartile from that of the fourth quartile. The data seem to indicate that the chief weakness of the contestants represented in the fourth quartile was their inability to place a correct score on the flavor of the product being judged. In criticizing the flavor of the products, there was little difference between contestants of the first and fourth quartiles. This is indicated by the fact

TABLE 5
Difference in scores between 1st and 4th quartiles

Product	Score card items									
	Flavor		Body & texture		Color		Sediment		Container & closure	
	Score	Criticism	Score	Criticism	Score	Criticism	Score	Score	Criticism	Score Criticism
Butter	7.39	1.95	3.39	3.25
Cheese ...	8.92	1.43	5.11	2.56	1.06	0.96
Milk	13.80	1.46	4.03	0.46	1.04
Ice cream	4.87	1.47	4.71	4.54	4.27	0.26	0.30 0.26

that the fourth quartile lags behind only 1.95 points in butter, 1.43 points in cheese, 1.46 points in milk and 1.47 points in ice cream. A marked difference existed between the first and fourth quartiles when the sense of touch (body and texture criticisms) was involved. This is shown by the divergence of the scores of body and texture in butter, cheese and ice cream. Contestants of the fourth quartile lost as many points in scoring body and texture of ice cream as they did in scoring flavor. Likewise, they lost heavily in the scoring of body and texture in cheese. The ratio between the criticism of body and texture and its score is narrow. From the data available it is not possible to make a precise observation on this point. The solution to the difficulty may lie in devoting more time to the development of the sense of touch.

Effect of range of score on contestant rating. It has been alleged that contestants, when in doubt as to the correct score, tend to judge an item conservatively. Official judges sometimes are criticized similarly. Data in table 6 indicate that official judges used about 75, 57, 63 and 89 per cent of

TABLE 6

Range in scores of officials and contestants in scoring flavor of dairy products

Class	Range of score used in scoring flavor of							
	Butter (8) ^a		Cheese (7) ^a		Milk (15) ^a		Ice cream (9) ^a	
	Range	%	Range	%	Range	%	Range	%
Official	6.0	75.0	4.0	57.1	9.5	63.3	8.0	88.9
1st individual.....	5.0	62.5	4.0	57.1	15.0	100.0	3.5	38.9
2nd "	5.0	62.5	4.5	64.3	6.5	43.3	3.5	38.9
3rd "	5.0	62.5	5.0	71.4	10.0	66.7	3.0	33.3
1st quartile.....	5.5	68.8	4.5	64.3	9.9	66.0	4.4	48.9
2nd "	5.2	65.0	3.8	54.3	9.6	64.0	4.2	46.7
3rd "	5.2	65.0	3.6	51.4	8.0	53.3	5.0	55.5
4th "	5.3	66.3	5.3	75.7	9.4	62.7	4.7	52.2

^a Normal range in flavor score for the product.

the normal range in scoring the flavor of butter, cheese, milk and ice cream, respectively.

In the scoring of butter for flavor, contestants of the first quartile used a slightly greater percentage of the normal range than did contestants of the remaining quartiles. The three highest individuals used a very slightly lower proportion of the normal range than did the average of the four quartiles.

The official judge of cheese was more conservative in the use of the score range of flavor than the judges of the other products. The ranking individual used the same range. Contestants making up the second and third quartiles were even more conservative. On the other hand, individuals composing the fourth quartile used about 76 per cent of the recommended range.

TABLE 7

Range in scores of officials and contestants in scoring body and texture of cheese and ice cream

Class	Range of score used in scoring body and texture of			
	Cheese (3.5) ^a		Ice cream (4.5) ^a	
	Actual range	% of normal range	Actual range	% of normal range
Official	3.5	100.0	2.0	44.4
1st individual.....	2.0	57.1	1.5	33.3
2nd "	2.5	71.4	1.5	33.3
3rd "	3.0	85.7	1.0	22.2
1st quartile.....	2.2	62.8	1.8	40.0
2nd "	2.3	65.7	1.6	35.5
3rd "	1.8	51.4	1.6	35.5
4th "	2.4	68.5	1.5	33.3

^a Normal range in body and texture score for the product.

In scoring milk for flavor, most of the contestants used about the same range as the official judge. The winning contestant used the entire normal range. This contestant was not outstanding in determining the flavor defects, but he did a superior job in evaluating the flavor defect. The contestant winning second place in the scoring of milk used less than half the percentage of the normal range used by the first place winner, yet this contestant lost 10.5 more points in evaluating flavor than did the individual placing first.

All students, regardless of quartile or individual standing, seemed to be unusually moderate in the scoring of the flavor of ice cream. The official judge utilized approximately 89 per cent of the normal range. Most of the contestants used one-third to one-half of the recommended range.

Contestants were more conservative than the official judge in using the recommended range for body and texture of cheese (table 7). The official judge of cheese used 100 per cent of the normal range. The official judge and all of the contestants were extremely conservative in using the recommended range of body and texture of ice cream.

GENERAL DISCUSSION

The score cards as set up for the judging of butter, cheese, milk and ice cream contain some items which do not test proficiency in judging. This has been recognized for several years. The fact that they do not test proficiency is no indication that the items should be eliminated. For example, data show that most participants judge bottle and cap about as well as the winning contestants. Deletion of this item might result in paying too little attention to the featuring of a clean and attractive bottle. A knowledge of the proper scoring of items of lesser importance is part of a student's training and should be retained. It is hoped that the time will come in the training of students when the item of flavor, for example, may be judged with less difference between the grades of the higher and lower ranking contestants than exists at present. When that time comes, the training in dairy products judging will be more effective.

In studying the data presented, the reader should keep in mind that the products used in the 1947 Collegiate Students' International Contest in the Judging of Dairy Products may have been of such a character that utilization of the entire normal range was not feasible, because the products may not have merited different scores. It should be emphasized that in any contest the "normal range" need not necessarily be used.

SUMMARY

1. The key to the success of the winning contestants lay in their abilities to evaluate the flavor of the dairy products more nearly in line with the judgment of the official than their competitors. Also, they approximated

more nearly the judges' decision in scoring and determining the body and texture of Cheddar cheese and vanilla ice cream. The three high contestants agreed that no body and texture defects were present in the butter samples scored.

2. The items of melting quality and color of ice cream, sediment, container and closure of milk, color and salt of butter, and color of cheese are not so important as flavor and/or body and texture in testing the judging proficiency of the participants in the contest.

3. With the exception of ice cream, contestants of the fourth quartile failed to score higher because of their inability to place a correct score on the flavor of the product.

4. Concerning the use of the suggested normal range in the scoring of each product, the following tendencies were observed: In the scoring of the flavor of butter, the contestants were more conservative than the judges. In the rating of the flavor of cheese and milk, the contestants and the judges were equally moderate. In the scoring of the flavor of ice cream, the contestants were much more conservative than the judge. All contestants were exceedingly cautious in placing scores on the body and texture of cheese and ice cream.

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MANOMETRIC MEASUREMENT OF THE GAS DESORBED FROM VACUUMIZED WHOLE MILK POWDER

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Packaging whole milk powder in an atmosphere extremely low in oxygen is recognized as being a safeguard in the prevention of off-flavors caused by oxidation of milk constituents. During spray drying of milk, gas is entrapped in the particles. This gas is not entirely removed by the ordinary single-stage gas-packing process. Therefore, packing milk powder in an atmosphere extremely low in oxygen becomes a problem of control of occluded oxygen, and possibly adsorbed oxygen, as well as one of proper technique in gas packing.

The origin of the entrapped gas has not been established definitely. Hetrick and Tracy (3) suggest that perhaps the oxygen dissolved in the milk is a factor in the amount of oxygen entrapped in the powder, but Coulter and Jenness (1) were unable to eliminate the entrapped gas in the powder particles by deaerating the condensed milk before drying.

The composition of the entrapped gas varies somewhat with the storage time of the dried milk in an atmosphere of air, according to Haller and Holm (2). Their results showed that the gas entrapped in the particles contained from 22 to 39 per cent oxygen.

Measurement of the amount of oxygen occluded has been done in a number of ways. Lea *et al.* (4) have derived a formula by which they calculate the milliliters of oxygen per gram of powder, knowing the initial per cent oxygen and the final per cent oxygen after equilibrium has been reached in the headspace gas of nitrogen-packed powder. Haller and Holm (2) used an apparatus with which they could remove the "sorbed" gases, measure their volume, and determine their composition.

If whole milk powder is put into a container and the container evacuated, the amount of gas that is removed will depend upon the temperature of the powder, the absolute pressure, the length of time of vacuumizing, as well as the physical characteristics of the powder itself. The occluded oxygen is not removed to any appreciable extent unless vacuumizing is continued for long periods of time. Use was made of this fact in this particular study to determine the amount of oxygen or gas entrapped in the powder.

The objective of this study was to measure the rate of desorption, the amount of gas held, and the composition of the gas "desorbed" from vacuumized whole milk powder by the manometric method. The results secured

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by this method also were compared with those secured by the method of Lea *et al.* (4). Using the manometric method, the effects of aging the powder in the air before vacuumizing and of saturating the milk with carbon dioxide before drying on the amount of gas held during vacuumizing, and on the composition of the desorbed gas, was determined. It was hoped that by this procedure information on the origin of the entrapped gas could be secured.

EXPERIMENTAL METHODS

The whole milk powder used in this study was prepared in the laboratory by drying preconcentrated milk in the small pilot drier. The drier was a pressure-spray type with a capacity of approximately 30 lb. of powder per hour. The apparatus shown in figure 1 was used to measure the

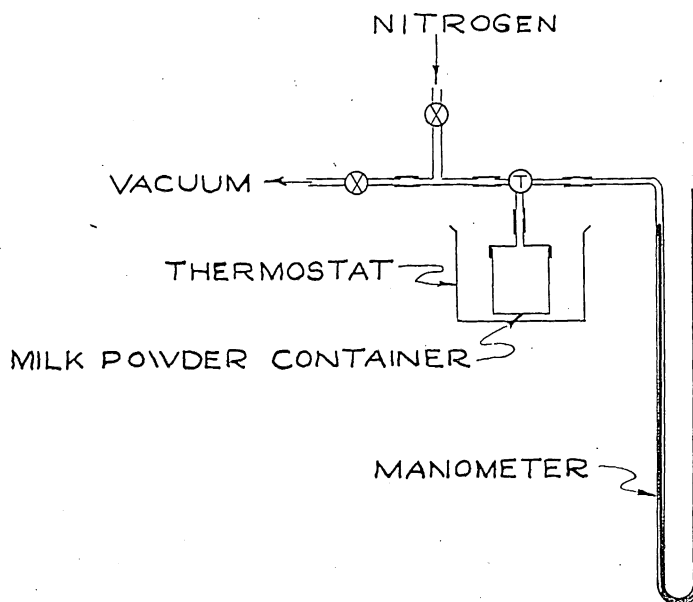


FIG. 1. Apparatus used to measure the gas desorbed from vacuumized whole milk powder.

rate of desorption and the amount of gas held. The composition of the gas desorbed from the vacuumized whole milk powder was determined by the method of Van Slyke and Sendroy (5). The whole milk powder container shown in the figure was calibrated up to the stopcock so that the packing density could be determined when the weight of milk powder was known. Capillary tubing was used for the connections and also for the manometer.

Whole milk powder was weighed into the cans so that the packing density in grams per milliliter of container volume was approximately 0.5. The manometer was affixed to the can and a three-way valve was set in such a way that the vacuum pump and the manometer both were connected

to the can of powder. By means of the vacuum pump, a vacuum was drawn on the system in approximately two minutes to an absolute pressure of 1-2 mm. mercury. The three-way cock then was turned in such a way that the can of powder was opened only to the manometer, and the vacuum pump was removed. As the entrapped air desorbed from the powder, the pressure increased. Absolute pressure measurements were made until the pressure became constant. All pressure measurements were made at 25° C. When the pressure measurements became constant, it was assumed that equilibrium conditions had been reached. Knowing the equilibrium pressure, the volume of the can, the weight of powder, and using a value of 1.31 for the density of air-free powder, the number of moles of gas, or volume of gas (*N.T.P.*) per gram of powder, which was held during the initial vacuumizing process, could be calculated. For this purpose, the following formula was developed.

$$\text{cc. gas/g.} = \left(\frac{22,400 P_e}{W R T} \right) \times \left(V_c - \frac{W}{1.31} \right)$$

Where P_e = the equilibrium pressure (mm. Hg)

W = " weight of powder in grams

V_c = " volume of the container (cc.)

T = " absolute temperature

R = " gas constant (62,400 cc. mm.)

1.31 = " assumed density of air-free milk powder in grams/cc.

When the equilibrium pressure was reached, the volume of gas was so small that a sample could not be removed for analysis. In order to determine the composition of the gas, nitrogen was admitted rapidly to a known pressure and then a sample of the mixed gas was removed for analysis. Blank determinations were made in which there was no milk powder in the can to determine the per cent oxygen in the nitrogen used. Knowing the per cent oxygen in the nitrogen used, the pressure of the nitrogen, the equilibrium pressure of the gas in the container, and the total pressure and the per cent oxygen of the gas in the container after the nitrogen was admitted, the percentage of oxygen in the gas that was desorbed from the sample of milk powder could be calculated by means of the following formula:

$$X_e = \frac{(X_T P_T) - (X_N P_N)}{P_e}$$

Where X_e = the per cent oxygen in the "desorbed" gas

X_T = the per cent oxygen in the mixed gas

P_T = the total pressure (mm. Hg)

X_N = the per cent oxygen in the nitrogen

P_N = the nitrogen pressure (mm. Hg)

P_e = the equilibrium pressure (mm. Hg)

It is recognized that this method is not an absolute one for measuring the amount of oxygen held by the particles during the vacuumizing process.

It does not include the adsorbed gas which may be present under equilibrium conditions. However, it is believed to show differences among different treatments of milk powder with respect to the amount of oxygen held during vacuumization.

RESULTS

Reproducibility of the method. In order to ascertain how closely values from a number of runs would agree, four determinations on the same powder were made. The results are shown in table 1. The absolute pressure measurements were reproduced closely. The same milk powder used

TABLE 1
Reproducibility of pressure measurements during desorption of gas from vacuumized whole milk powder

Time after vacuumizing (hr.)	Absolute pressure ($P_B - P$) at 25° C. (mm. Hg)			
	Trial 1	Trial 2	Trial 3	Trial 4
0	1	1	2	2
18	21	21	21	20
27	28	28	28	28
42	30	31	30	30
55	32	31	31	31
71	34	33	33	33
90	35	34	34	34
98	35	35	35	35
114	35	35	35	36
144	36	35	36	36
187	37	36	37	37 ^a
258	37	36	40	62
336	40	41	40	10
403	41	42	42	11
474	41	41	41	11

^a Leaker re-evacuated to 2 mm.

in this experiment also was packed in nitrogen gas and gas analyses were run on the headspace gas of the samples of powder until equilibrium was reached. The formula developed by Lea *et al.* (4) was used to calculate the milliliters of oxygen per gram of powder, as well as the milliliters of gas per gram of powder, and the values were compared to those secured by the manometric method. The results are listed in table 2.

From these results it can be seen that the two methods of determination check closely with respect to the milliliters of oxygen per gram, but not so well for the milliliters of gas per gram. The conclusion to be reached is that perhaps the difficulty lies in the composition of the gas desorbed with respect to per cent oxygen. Lea *et al.* (4) have assumed in their derivation that the gas entrapped in the particles contains 20.85 per cent oxygen. If the gas desorbed has a higher oxygen content, as data by the manometric method show, then one would expect to have a lower value for the milli-

liters of gas evolved per gram of powder. The assumption that the composition of the gas entrapped in the powder is 20.85 per cent oxygen is not significant when one uses this assumption to calculate the milliliters of oxygen per gram of powder. With the formula that they have derived, any error involved in this assumption would largely cancel out. However, in the calculation of the milliliters of gas held per gram, the value for the per cent oxygen in the gas desorbed becomes important.

With the manometric method, the values for the milliliters of gas per gram are more accurate than those representing the milliliters of oxygen per gram because the pressure can be measured accurately, but small variations in the per cent oxygen in the mixed gas make large errors in the

TABLE 2

A comparison of the manometric method and the method of Lea et al. (4) for determination of ml. of oxygen and ml. gas entrapped per gram of whole milk powder

Manometric method									
Trial	Vol.	W	P _e	P _N	P _T	%O ₂ (P _T)	%O ₂ gas desorbed	Ml. gas/g.	Ml. O ₂ /g.
1	477	238.5	43	712	755	2.21	33.8	0.064	0.0216
2	474	237.0	43	710	753	2.38	36.7	0.064	0.0235
Method of Lea et al.									
Time (days)	% O ₂	Equilibrium % O ₂	D	d	ml. gas/g.	ml. O ₂ /g.			
0	0.3			
4	2.01	2.19	1.31	0.50	0.113	0.0235			
7	2.24			
14	2.16			
26	2.33			

milliliters of oxygen. A difference of approximately 0.1 per cent oxygen in the gas mixture makes a difference of about 1.8 per cent in the oxygen content of the desorbed gas. However, the data are sufficiently accurate to show that the per cent oxygen in the desorbed gas is higher than that of ordinary air. This is in agreement with the results of Haller and Holm (2). One could speculate from this that the source of the entrapped air in the particles may be the gas that is dissolved in the milk before drying, or that oxygen is preferentially held in the cavities of the particle during vacuumizing.

Importance of gas entrapped in milk at time of drying. If the origin of the gas which is entrapped in the milk powder particles is the milk before drying, it was reasoned that the composition of the desorbed gas could be changed by changing the composition of the gas dissolved in milk. Two lots of powder were dried, one serving as a control and one in which

the carbon dioxide was bubbled into the condensed milk before drying. Both lots were spray dried as nearly alike as possible as far as pressures, temperatures, and nozzle sizes were concerned. Duplicate tests were run on both lots of powder when the powder was freshly prepared, and another set of duplicate runs was made after the powder was exposed to air for 24 hours after drying. The data for these runs are recorded in table 3

TABLE 3

Effect of saturating the condensed milk with carbon dioxide and age of powder before vacuumizing on the gas content and composition of the gases desorbed from vacuumized whole milk powder

Sample no.	Trial	Treatment	Vol. ^a	W	P _e	P _N	P _T	% O ₂ (P _T)
1	A	Control, packed immediately	477	238.5	29	721	750	1.75
	B	Leaker
2	A	Control, packed 24 hr.	474	237	35	843	878	1.62
	B		475	237	35	853	888	1.54
3	A	CO ₂ added to milk, packed immediately	474	202	58	812	870	1.34
	B		479	210	57	800	857	1.20
4	A	CO ₂ added to milk, packed after 24 hr.	468	200	61	819	880	2.10
			469	200	62	808	870	2.06

Sample no.	% O ₂ desorbed gas	% CO ₂ (P _T)	% CO ₂ desorbed gas	Ml. gas/g.	Ml. O ₂ /g.	Ml. CO ₂ /g.
1	37.5	0.043	0.0162
	Leaker
2	33.4	0.052	0.0174
	32.1	0.053	0.0170
3	15.9	2.61	39.1	0.110	0.0175	0.0430
	13.8	2.75	41.3	0.104	0.0143	0.0429
4	26.3	2.19	31.6	0.116	0.0305	0.0367
	24.9	2.16	30.4	0.118	0.0294	0.0359

^a Vol. = Volume of container (cc.).

W = Weight of powder in grams.

P_e = The equilibrium pressure (mm. Hg).

P_N = Pressure of nitrogen (mm. Hg).

P_T = Total pressure (mm. Hg).

and the pressure changes by the manometric method are recorded in figure 2. The per cent oxygen in the air desorbed from the control powder again was higher than that in ordinary air. On aging the control powder before vacuumizing, the per cent oxygen in the gas desorbed decreased slightly, but the total volume of oxygen held per gram as well as the total gas per gram increased by this treatment. Similar results on the per cent oxygen in the air desorbed were secured by Haller and Holm (2). When powder is gas-packed after aging in air for 24 hours, the per cent oxygen in the headspace gas increases over that in powder gas-packed immediately after drying (1, 3). The results of this work seem to show that even

though the per cent oxygen in the desorbed gas decreases by aging the powder 24 hours before vacuumizing, the total amount of oxygen actually increases because the total amount of gas increases proportionately more than the per cent oxygen decreases.

The effect of milk and powder treatment on composition of desorbed gas and amounts of gas held by powder. When powder was vacuumized immediately after drying, the total volume of gas held per gram of powder was more than doubled by bubbling carbon dioxide into the condensed milk before drying. The composition of the desorbed gas also was altered. This would lend support to the belief that at least in part the air or gas

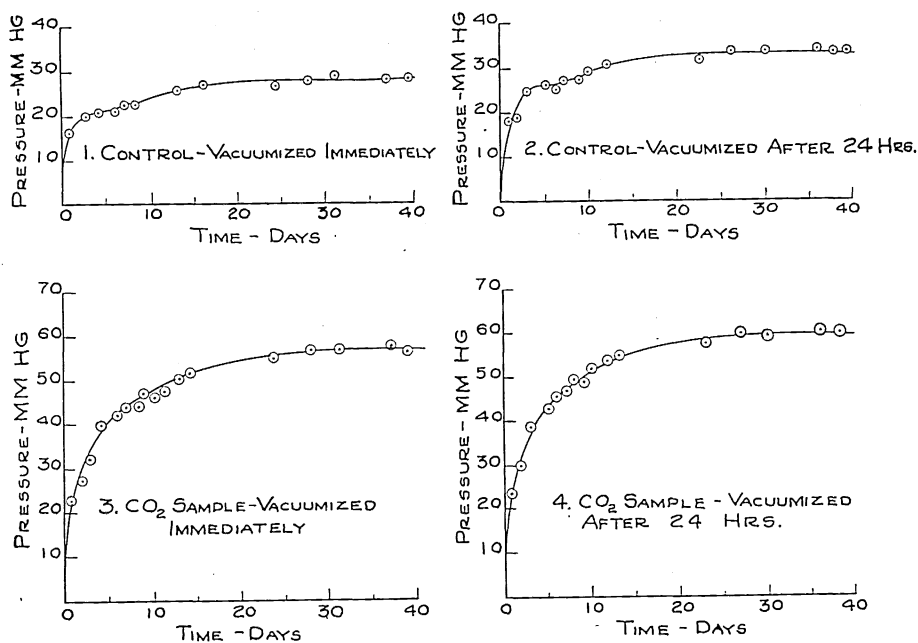


FIG. 2. Pressure changes during desorption of gas from vacuumized whole milk powder.

dissolved in milk is a source of the gas entrapped in the powder particles. Even though the total volume of gas held per gram by this treatment was much higher than in the control powder, the volume of oxygen held per gram was nearly the same as the control batch. When powder manufactured from condensed milk that had been saturated with carbon dioxide before drying was exposed to air for 24 hours before vacuumizing, a slight increase in total gas held per gram of powder was observed and almost a two-fold increase in the amount of oxygen held per gram was found. The carbon dioxide content of the powder was decreased by exposing this powder to air for 24 hours before vacuumizing. The increase which was observed in the total gas content by exposing milk powder 24 hours before

vacuumizing could be attributed to temperature and pressure phenomena, because the powder which was vacuumized immediately was warm and as the powder cooled, it is likely that more gas would be sorbed.

SUMMARY

A manometric method is presented for the determination of the amount of gas held and the composition of the gas desorbed from vacuumized whole milk powder. The amount of sorbed gases in dried milk varied with the methods of preparation and packaging. When freshly made powder was vacuumized after drying, the per cent oxygen in the gas desorbed from powder was higher than that of ordinary air. Upon exposure of powder to air for 24 hours before vacuumizing, the per cent oxygen in the gas desorbed was less, but not lower than the per cent oxygen in normal air. The total volume of oxygen held per gram of powder was increased by exposure of powder to air for 24 hours before vacuumizing. The composition of the gas desorbed and the total quantity of gas per gram of powder could be varied by saturating the milk with carbon dioxide before drying. This would lend support to the belief that, at least in part, the source of the gas entrapped in powder is the gas dissolved in milk.

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THE EFFECT OF AGITATION UPON THE LIVABILITY OF BOVINE SPERMATOZOA¹

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In the routine operation of most artificial breeding associations, diluted semen samples are transported from the central bull stud to the outlying inseminators, and often considerable distances are involved. Various means of transportation are employed and it is obvious that the semen samples are subjected to different amounts of agitation enroute.

Bretschneider (2) reported that the vigorous shaking of bull semen for 3 minutes destroyed motility. Spermatozoa from the normal ejaculate showed more resistance to shaking than did those secured from the testicle or epididymis. Smirnov-Ugrjumov (14) observed that the transportation of undiluted bull semen in thermos flasks at 15–20° C. for distances of 0.6 to 5.9 miles brought about a reduction in spermatozoan activity. Hronopulo (6) reported that transportation of undiluted bull semen did not affect the fertility of the semen during the first 4 hours of storage. After a storage period of 4 hours, however, the fertility of semen transported distances greater than 21.7 miles was markedly reduced. Ayyar (1) noted that hand shaking during transport killed bull spermatozoa.

Several workers have noted effects of agitation in connection with studies of semen physiology. Gunn (4) reported that periodic shaking of ram semen contained in rubber-stoppered test tubes was effective in providing the aeration which he considered necessary for maintenance of spermatozoan motility. Motility was maintained even when shaking was vigorous. On the other hand, Nagorny and Smirnov (11) found that the resistance of ram spermatozoa to sodium chloride was decreased by continuous agitation. During the course of metabolism studies with ram and bull semen, Mann (10) observed that when a suspension of spermatozoa was shaken vigorously in the presence of air, the cytochrome enzyme within the cell was oxidized rapidly.

In the process of examining samples of diluted semen shipped to this laboratory from the several artificial breeding cooperatives in Pennsylvania, it was

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noted that the motility of the spermatozoa usually was lower in tubes of diluted semen shipped partially filled than in completely filled tubes. Poor livability often was evident, particularly in samples shipped in 14-ml. capacity test tubes which contained only 3-4 ml. of diluted semen. The decrease in semen quality observed may have been due in part to harmful effects of agitation in transit. Thus, the present study was proposed to determine the effect of mechanical agitation of diluted bull semen upon the livability of the spermatozoa.

EXPERIMENTAL PROCEDURE

Fifteen semen samples were obtained by means of an artificial vagina from three fertile bulls of the College dairy herd representing the Ayrshire, Brown Swiss and Holstein breeds. The 15 ejaculates were diluted at a constant rate of one part of fresh semen to 24 parts of egg yolk-citrate diluter. The diluter was composed of one part of fresh egg yolk and one part of citrate buffer prepared by dissolving 3.6 g. of sodium citrate dihydrate in 100 ml. of water distilled over glass.

The effect of agitation upon spermatozoan livability was studied by subjecting diluted semen in three series of test tubes to mechanical agitation for 6, 12 and 24 hours. A fourth series received no mechanical agitation and served as the controls. Each of the series consisted of four test tubes (15 × 125 mm.), which contained 3.5 ml., 7.0 ml., 10.5 ml. and approximately 14.0 ml. of diluted semen. With these amounts of material the tubes were approximately one-quarter filled, half filled, three-quarters filled and filled to the bottom of the cork stopper. This experimental design made it possible to determine not only the effect of varying amounts of agitation but also the effect of agitation upon the livability of spermatozoa in test tubes containing different volumes of semen.

The test tubes were prepared by placing the desired volume of diluted semen in each sterile tube by means of a sterile pipette. The tubes were stoppered with sterile corks, and melted paraffin was applied to the juncture of cork and glass to complete the closure. The tubes of diluted semen were placed in a water bath at room temperature and gradually cooled to about 7° C. in a mechanical refrigerator. In order to maintain a temperature of from 5 to 10° C. during agitation and at the same time simulate field shipping conditions, each series of tubes to be agitated was packaged in refrigerated cardboard cartons. Methods and materials as described by Perry (12) were used in packaging the samples. Refrigeration was provided by 800 g. of ice contained in rubber balloons. A test tube containing water at 5° C. was packaged next to the tubes of diluted semen. When the cartons were opened, the temperature was determined by inserting a cooled thermometer (5° C.) into the tube of water. The control tubes of diluted semen were not packaged and were stored in a refrigerator at 5° C.

Agitation was provided by placing the shipping cartons on a mechanical agitator which operated at the rate of 76 oscillations per minute through a horizontal distance of 4 inches. The cartons were placed on the agitator so that the longitudinal axis of the test tubes was parallel to the horizontal axis of the

agitator frame. Following the prescribed period of agitation, the cartons were opened and the temperatures of the contents of those cartons subjected to 24 hours of agitation were determined. The samples then were placed in a 5° C. water bath and stored in a refrigerator maintained at that temperature.

In addition to motility estimations made before and after agitation, estimations were made every 2 days during the 20-day storage period. In order to minimize bias on the part of the observer making the motility estimations, randomized numbers were placed on the test tubes prior to agitation.

RESULTS

The 15 semen samples studied had a mean concentration of 1,141,000 spermatozoa per cubic millimeter, a mean initial motility of 63 per cent active spermatozoa and a mean methylene-blue reduction time of 12 minutes. The mean temperature to which the diluted samples were cooled prior to packaging was 7.1° C. and ranged from 5.5 to 8.9° C. The mean temperature of the samples after 24 hours agitation was 6.8° C., with a range of from 5.5 to 8.9° C.

The mean percentages of motile spermatozoa during 20 days of storage are presented in table 1. Each figure represents a mean of 15 ejaculates. Mechanical agitation of the partially filled tubes brought about a significant reduction in spermatozoan livability. The decrease in livability was related directly to the length of the agitation period. In addition, it was noted that the effect of agitation was related to the volume of semen contained in the tubes. Thus, the ability of the spermatozoa to remain motile during storage following agitation for 6, 12 and 24 hours was less in the one-quarter filled tubes than in the half filled tubes and less in the half filled than in the three-quarters filled tubes. When the tubes were completely filled, spermatozoan livability was not affected, as

TABLE 1
Effect of mechanical agitation upon the livability of bull spermatozoa

Fullness of test tube	Length of agitation (hr.)	Per cent motile spermatozoa (15 ejaculates)					
		Before storage	After storage at 5° C. for				
			4 days	8 days	12 days	16 days	20 days
Filled	0	63	49	43	32	17	5
	6	63	52	42	31	16	7
	12	63	50	41	29	16	7
	24	63	51	42	30	15	5
Three-quarters filled	0	63	51	39	28	17	7
	6	63	47	32	23	9	3
	12	63	43	31	16	6	3
	24	63	41	27	13	6	1
Half filled	0	63	51	42	26	13	7
	6	63	41	29	19	6	1
	12	63	38	27	13	4	0
	24	63	36	19	11	4	1
One-quarter filled	0	63	49	26	13	5	3
	6	63	39	21	9	3	0
	12	63	31	21	11	3	1
	24	63	27	10	5	2	1

markedly by agitation. Differences in livability of the spermatozoa also were obtained in the controls which received no mechanical agitation. Spermatozoa in the completely filled tubes maintained a significantly higher level of motility during storage than spermatozoa in one-quarter filled tubes.

Analysis of variance (table 2) involving 2,400 motility observations showed highly significant differences ($P = < 0.01$) between the two treatments, length of agitation (L) and fullness of tube (F), as well as between ejaculates and storage intervals. The interaction of the two treatments ($L \times F$) also was found to be highly significant, as were all other first and second order interactions.

TABLE 2
Analysis of variance of per cent motile spermatozoa during 20 days of storage following agitation for 0, 6, 12 and 24 hours

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	2,399	9,724	
Length of agitation (L)	3	304	101.33 ^a
Fullness of tube (F)	3	708	236.00 ^a
Storage intervals (S)	9	5,371	596.78 ^a
Ejaculates (E)	14	1,588	113.43 ^a
Interactions:			
$L \times F$	9	63	7.00 ^a
$S \times F$	27	100	3.70 ^a
$S \times L$	27	40	1.48 ^a
$L \times E$	42	42	1.00 ^a
$F \times E$	42	75	1.79 ^a
$S \times E$	126	437	3.47 ^a
$L \times F \times E$	126	138	1.10 ^a
$S \times L \times E$	378	171	0.45 ^a
$S \times L \times F$	81	62	0.77 ^a
$S \times F \times E$	378	225	0.60 ^a
* Remainder	1,134	400	0.35

^a = Significant at the 1 per cent level.

According to the least mean differences required for significance, differences in livability between the partially filled tubes and the filled tubes were highly significant after 6, 12 and 24 hours of agitation. The differences between the filled tubes of semen which received no agitation and those agitated for 6 and 12 hours were not statistically significant. However, after agitation for 24 hours, the differences barely reached significance at the 5 per cent level. Highly significant differences were found between the one-quarter filled and the completely filled tubes which were not subjected to mechanical agitation, while the differences between the filled tubes and the half and three-quarters filled tubes were not statistically significant.

DISCUSSION

The present study was designed to determine the effect of agitation upon the livability and metabolism of bovine spermatozoa. It was hoped that the latter information would be useful in explaining results obtained in the livability phase. Because of difficulties in obtaining reliable results with the methods employed

in the metabolism study, this phase of the problem was not completed. However, it is possible that the detrimental effect of mechanical agitation upon spermatozoan livability is related to the amount of atmospheric oxygen in the test tubes.

The results of the livability study showed that irrespective of the length of agitation an inverse relationship existed between the amount of air in the test tubes and the livability of the spermatozoa. However, decreases in livability were greatest in those tubes which contained the largest volumes of air and which were agitated for the longest periods of time. When a minimum of air was present (filled tubes) agitation did not affect markedly the livability of the spermatozoa. These observations of the detrimental effects of aeration are supported by the statistical analysis of these data. As shown in table 2, all of the sources of variation were found to be highly significant. However, the table shows that a greater mean square was obtained for fullness of tube than for length of agitation. The mean square for the interaction, length of agitation \times fullness of tube, also was larger than the mean square for any of the other first order interactions.

It has been shown (3, 8, 13, 16 and others) that although a certain volume of oxygen normally is utilized in the metabolism of bovine spermatozoa, respiration is not essential for motility (7). While the exact role of oxygen in the metabolic processes is not clear, there is evidence that an excess of oxygen, in certain instances, may be detrimental to spermatozoan livability. Walton (15) concluded that protection of semen against exposure to air may be beneficial to livability and recommended that the semen be covered with a layer of medicinal paraffin oil. Willett and Salisbury (17) also found that motility was maintained longer during storage when semen was covered with a layer of mineral oil. MacLeod (9) found that oxygen was detrimental to the motility of human spermatozoa. Recently Salisbury (13) reported that bovine spermatozoa in low concentration were harmed by oxygen. Based on these findings, it is possible in the present study that excess aeration of the diluted semen was responsible, in part, for the decreases in livability obtained.

CONCLUSIONS

1. In the routine shipment of partially filled tubes of diluted bull semen a decrease in spermatozoan livability may be encountered due, in part, to the effect of agitation.
2. The harmful effect of agitation may be minimized by completely filling the test tubes with diluted semen.
3. On the basis of the data obtained in this experiment, it seems advisable to ship diluted semen in completely filled test tubes of different capacities to meet the individual requirements of the inseminators.

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HEAT INACTIVATION OF MILK PHOSPHATASE IN DAIRY PRODUCTS

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A description of the phosphatase test and the modifications that make it applicable to various dairy products to determine the adequacy of pasteurization was published in 1947 (12). In a later publication (13), it was pointed out that negative results with the phosphatase test indicate that the pathogenic organisms that may have been present were destroyed.

With a phosphatase test available that is quantitative over a wide range (from no heating to complete inactivation) and that is relatively sensitive and precise, and with considerable data available in the literature on the thermal death points of various microorganisms, it seemed desirable to determine experimentally the heating conditions necessary to produce various degrees of inactivation, including complete inactivation, of the phosphatase enzyme in milk and in some other dairy products. Such results would be useful in formulating pasteurization standards for various dairy products besides milk.

This report describes a laboratory pasteurizer that was used in these studies for controlling the temperature and duration of heating with a high degree of precision, and presents the results of phosphatase-inactivation experiments on whole milk, skim milk, cream (20 and 40 per cent fat content), ice cream and sherbet mixes, Cheddar cheese and cheese mixtures with emulsifiers or various other substances added.

Precise control of the temperature and duration of heating is necessary for reliable results in studying experimentally the thermal destruction of bacteria and phosphatase. North and Park (10), determining thermal death points of tubercle bacilli, used a laboratory pasteurizer fitted with a tubular metal coil immersed in a bath at a controlled temperature. The milk, heated to the desired temperature, was inoculated and was allowed to flow by gravity into the tubular coil where it was held at the experimental temperature. This method offered relatively more precise results than older methods, in which samples, at room temperature, were placed in glass tubes, inoculated and the tubes then placed in the heated bath. It reduced the heat lag, allowed more precise control of the heating time, and eliminated surface cooling.

Some investigators, heating samples in glass tubes (11) or in metal containers (6, 8), used a series of three water baths—the first one at a temperature below the desired holding temperature in order to preheat the samples uniformly, the second one at a temperature somewhat higher than the holding temperature in order to decrease the time lag by heating the samples rapidly, and the third one at the desired holding temperature. Despite the need for increasing the temperature rapidly, there appears to be considerable possibility of over-heating some particles

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of the sample when any part of the equipment containing the sample is heated at a temperature higher than the specified experimental temperature.

Gilcreas and O'Brien (4) recently have described a laboratory pasteurizer, used in bacteriological experiments, fitted with an automatic inoculating and sampling device actuated by an electric timer. With this equipment, inocula can be injected into a medium that is held constantly at the desired temperature, and samples likewise can be withdrawn with time intervals controlled very accurately. Although the heating-time lag is thus greatly reduced, this equipment could not be used for obtaining phosphatase-test data, because in phosphatase experiments it is necessary to control the heating conditions of the entire sample rather than only the portion used as an inoculum in bacteriological experiments.

EXPERIMENTAL METHODS

Attempts were made at first to make use of a Mallory-type heater, with the heating tube surrounded by steam, but it was difficult to determine and control the temperatures of small samples with the desired precision without installing a highly sensitive thermocouple and without other modifications of the control of the temperature. The results of the phosphatase tests on the samples heated in this manner were not sufficiently consistent for this purpose.

A laboratory pasteurizer, illustrated in figure 1, was assembled and used for heating fluid samples. The pasteurizer comprised a thin-wall tubular metal coil 30 feet long and one-eighth inch internal diameter, with a metal holding chamber connected at the lower end. This assembly was immersed in a water bath fitted with heaters, stirrer and thermoregulator, which controlled the temperature of the bath with a variation not greater than $\pm 0.2^\circ$ F. The pasteurizer coil as used at first was fitted at the midway point with a T-tube connection (not shown in diagram) in which a four-junction thermocouple in thin-wall glass tubing was installed. A similar thermocouple was installed permanently through the stopper in the holding chamber. Temperatures were determined by means of a Leeds and Northrup type K potentiometer. Six mercury thermoregulators set at approximately 5° intervals between 142 and 168° F. were used. A special thermometer, reading 140 – 180° F. in 0.1° intervals, calibrated against one that had been checked at the National Bureau of Standards, was used in the bath and for calibrating the thermocouples. Heating-time periods were regulated with an electric stopclock calibrated in seconds.

To reduce the heating-time lag uniformly, the samples first were warmed in the phosphatase-test bath to 99 – 100° F., and then forced into the pasteurizer coil at high speed by means of air pressure. A suitable initial pressure, controlled by pressure regulator *A* (fig. 1) set at between 9 and 12 inches of mercury for milk and 12 to 15 inches for cream, was built up by means of a slow flow of air into flask *B*. Twenty to thirty-five ml. of warmed sample was put into sample chamber *S*₁, which was stoppered, and stopcock *SC* was turned to build up the pressure on the sample. Then rubber inlet tube *S*₂ was opened and the stopclock was started. The pressure forced the sample into and through pasteurizer coil *C* and into the bottom of holding chamber *HC*. The lower clamp on the inlet tube immediately

was placed below the water line in the bath and the inlet tube was closed. Under these conditions, the largest decrease of temperature that could be detected by means of the thermocouple at the midway point in the pasteurizer coil during flow was 0.2°F. , and such decrease was only momentary. No change of temperature in the holding chamber could be detected.

At the ends of specified time periods, 2- to 3-ml. test samples were withdrawn quickly from the holding chamber through narrow-bore glass tubes *D*, by means of suction, into test tubes immersed in ice water. To allow for lag, a correction of 3 seconds for the smaller samples and 4 seconds for the larger ones was subtracted from the total heating time from the beginning of flow. The heating times thus corrected and recorded in the graphs are believed to be the averages of the periods during which the entire sample of each product was held at the experimental temperature. This seems a more feasible time to record than the over-all heating time.

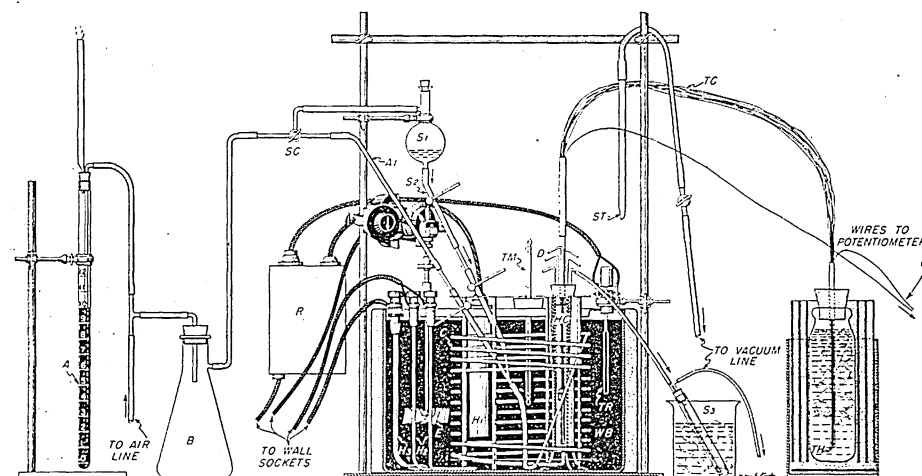


FIG. 1. Laboratory pasteurizer. *A*—adjustable air-pressure regulator. *B*—air-pressure reservoir flask, 1 liter. *SC*—three-way stopcock. *S*₁—sample chamber. *S*₂—sample inlet tube. *A*₁—air inlet tube for agitating sample. *C*—pasteurizer coil, 30 ft. long, $\frac{1}{8}$ in. internal diameter (tin alloy). *HC*—metal holding chamber, insulated above water line. *D*—glass tubes for removing samples. *S*₃—samples collected in tubes in ice water. *WB*—water bath. *H*—heaters, 300-watt: *H*₁ operated by thermoregulator, *H*₂ on constantly, *H*₃ for increasing bath temperature rapidly. *E*—stirrer. *TM*—thermometer. *TR*—thermoregulator. *R*—relay. *TC*—thermocouple, copper-constantan, 4-junction in series. *TH*—thermocouple reference junction in insulated ice bath. *ST*—suction tube for cleaning.

For the fluid samples that were heated at the higher temperatures and for the shorter periods, the time required to heat the entire sample was decreased by using only 20 ml. of sample and increasing the initial pressure to as high as 12 inches of mercury for milk and 15 inches for cream. Under these conditions it required not more than 4 seconds for the entire sample to pass into the pasteurizer coil and 6 seconds from the beginning of flow for the front end of the sample to reach the holding chamber.

To avoid the difficulty caused by the cream rising or a protective pellicle form-

ing on the samples, mentioned by Smith (14) and by North and Park (10), the samples were agitated by means of air. As soon as the sample had passed in and the clamp on the sample inlet tube S_2 had been closed, stopcock SC was turned and the clamp on air inlet tube A_1 was released, permitting air to flow slowly through the pasteurizer coil and to agitate the sample with two or three bubbles per second. The temperature of the air above the sample was found to be the same as that of the sample. The temperatures in the holding chamber and in the sample did not fluctuate as much as those in the water bath.

At least six test samples, each heated for a different period of time, were obtained of each product heated at each temperature. Sufficient test samples were obtained so that at least one was negative (zero value) and at least four were positive. If the results did not meet these conditions, the experimental heating was repeated at the same temperature but under a modified set of time conditions. For example (fig. 2), a 20-ml. sample of whole milk was heated at 158.2° F. and test samples were obtained first that were heated for 42, 50, 60, 75, 90, and 110 seconds, respectively; the first two yielded phosphatase values less than 5 units per ml. and the last four were negative. The heating was repeated and test samples obtained that had been treated for 24, 27, 30, 35, and 42 seconds; the first yielded more than 40 units and the last yielded less than 2 units per ml.

The milks tested were fresh and were taken from the composite milks obtained from a large herd. The tests on creams and skim milks were run on samples prepared by separating portions of the same whole milks that were tested. The ice cream mixes contained 15 per cent fat, 8.5 per cent milk serum solids, 14 per cent sugar, and 0.3 per cent stabilizer, and had an average pH of 6.22. The sherbet mix contained 4 per cent fat, 3.5 per cent serum solids, 25 per cent sugar, and 0.3 per cent stabilizer, and had a pH of 6.30 before pasteurization and the addition of acid. The fat and phosphatase present in the mixes were from the raw cream, additional serum solids being furnished by condensed skim milk. The Cheddar cheese, which was made from raw milk, was of normal composition and between 1 and 2 months old when tested. It had a pH value of 5.29 and a phosphatase value of 3,450 units per g. The original phosphatase values of the other products were normal and within the ranges stated earlier (13).

In addition to tests on fluid products, 3-g. samples of ground cheese were placed in heat-sealing metal foil envelopes, and these were pressed to a uniform thickness of 1 mm., warmed in the phosphatase-test bath and immersed in the pasteurizing bath. With a thermocouple coated with shellac and Miracle adhesive and sealed in with such cheese samples, the time required for the temperatures of the samples to reach within 0.25° F. of that of the bath at 150° F. was found to be approximately 18 seconds. A correction of 18 seconds was subtracted from the total heating time.

To determine the effects of pH on the inactivation of the enzyme by heat, 5-ml. portions of a sample of milk (a mixture of 10 per cent of raw milk with 90 per cent of pasteurized milk) were placed in a series of 12 test tubes, various quantities of normal acid or alkali were added—*i.e.*, from 0.35 ml. of acid to 0.1 ml. of alkali, yielding a step-wise pH range of 4.09 to 8.42—the pH values were

determined, the samples were funneled into clean tubes so that there was no liquid on the inner surfaces of the tubes above the samples, and they were placed in a rack, warmed to 99–100° F., and then heated with agitation in a water bath at 140° F. for 5 minutes. Two and one-fourth minutes additional time was allowed for the temperatures of the samples to reach 139° F. The samples were cooled in

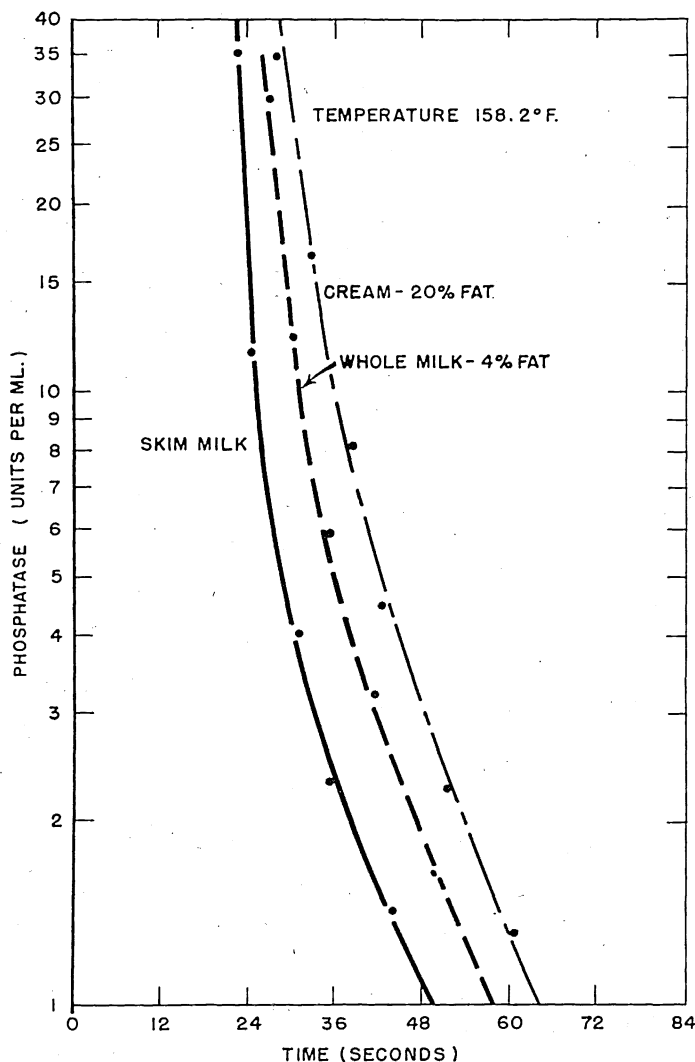


FIG. 2. Effects of duration of heating, at one temperature, on inactivation of phosphatase in skim milk, whole milk, and cream.

ice water, titrated back to the original pH with alkali or acid and tested for phosphatase activity. Corrections were made for the volumes of added acid and alkali.

Phosphatase tests were made by the method described earlier (12), and determinations of the intensity of the color were made with a Klett-Summerson photoelectric colorimeter with round matched tubes. Samples yielding less than 1 unit

per 1 ml. or per 0.5 g. of product in the test were considered negative, since such small values are difficult to determine accurately, even with a colorimeter, because of slight possible variations in the readings made on the controls.

RESULTS

The time-temperature inactivation results are summarized graphically. Figure 2 shows test data obtained on skim milk, whole milk, and 20 per cent cream, all were heated at 158.2° F., and illustrates the method of plotting the results. By conducting a series of tests on samples heated at a sufficient number of dif-

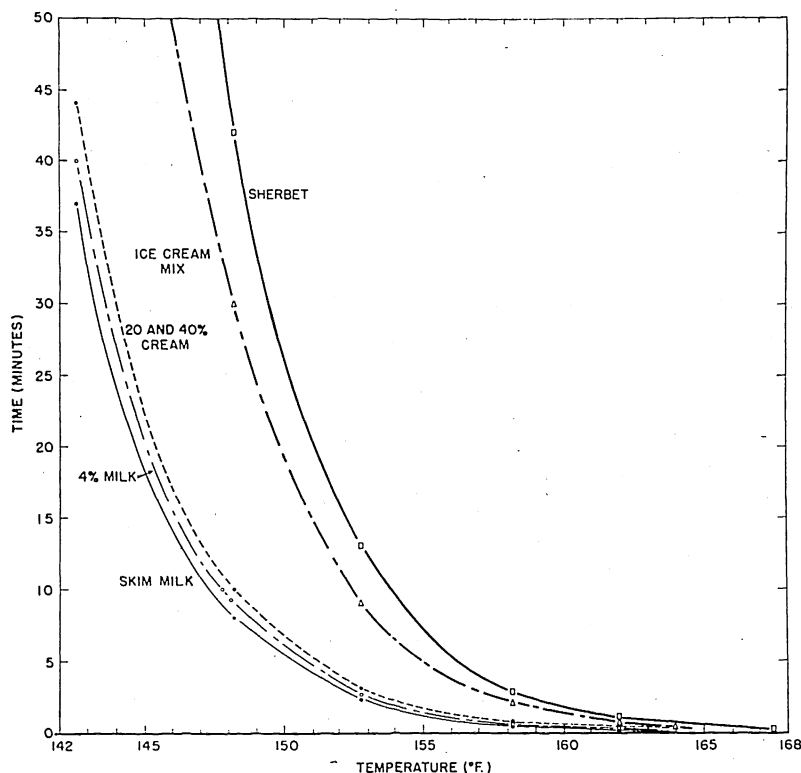


FIG. 3. Times and temperatures of heating required to reduce phosphatase activity to 2 units per 0.5 ml. (4 units per ml.) in different dairy products—test data plotted on an arithmetic scale.

ferent time periods, and thus plotting the data, it was possible to determine the heating times required at this temperature to produce zero values and also different degrees of inactivation.

With the phosphatase values plotted on a logarithmic scale and the duration of heating on an arithmetic scale (fig. 2), the curves in all experiments deviated from a straight-line course in a direction that indicates a marked decrease in the rate of inactivation as the time of heating is prolonged—*i.e.*, the rate of destruction of the enzyme by heat is most rapid at first and diminishes greatly with time. The curves prepared in this manner from data obtained at the lower temperatures

intersect the horizontal axis at a narrow angle, and the curves from data at the higher temperatures intersect the horizontal axis at a wide angle, showing that complete destruction is approached more slowly at low temperatures than at high temperatures.

By means of a similar series of experiments at each different temperature, data were obtained and plotted to determine the heating conditions just sufficient to reduce phosphatase activity to a value of four units per ml. in skim milk, whole milk, 20 and 40 per cent cream, ice cream mix, and sherbet mix. With data plotted arithmetically, as shown in figure 3, it is difficult to evaluate accurately the

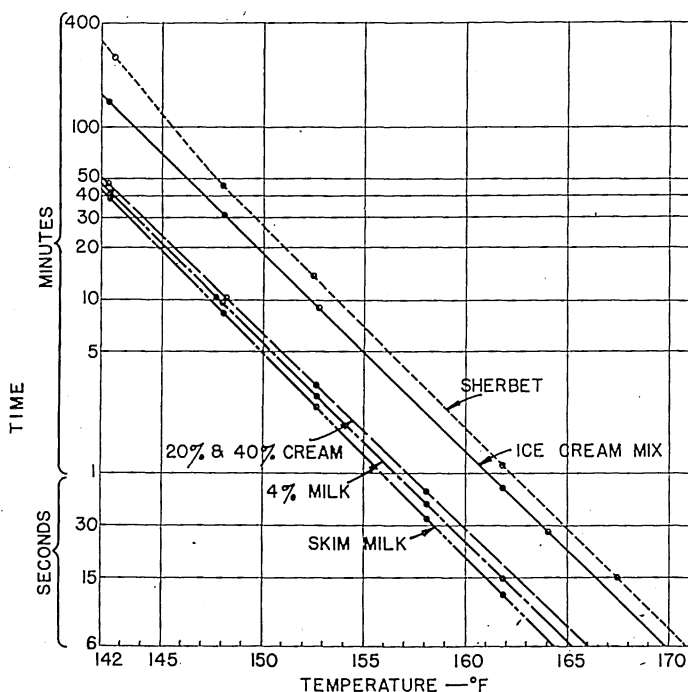


FIG. 4. Times and temperatures of heating required to reduce phosphatase activity to 2 units per 0.5 ml. (4 units per ml.) in different dairy products—data the same as in figure 3, but plotted on a semi-logarithmic scale.

heating times required in the high-temperature range. With the same data plotted on semi-logarithmic graph paper, however, as shown in figure 4, the points for each product form a straight line and the plot is readily usable.

The straight-line course of the time-temperature inactivation data conforms with results obtained earlier by Holland and Dahlberg (8) and Marquardt and Dahlberg (9) in studies on the effect of heat on cream layer volumes; with North and Park's results, as interpreted by Dahlberg (3), on the killing of tubercle bacilli; and with Hening and Dahlberg's (6) and Holland and Dahlberg's (8) results on the killing of *Escherichia coli*, the inactivation of phosphatase, and the effects on other properties. Mathematical equations pertaining to the straight-line pattern of time-temperature effects on a semi-logarithmic scale, produced in heat-

ing milk, were presented by Marquardt and Dahlberg (9) for reduction of cream layer volumes; by Sommer (15) for thermal death points, present pasteurization standards, and reduction of cream layer volumes; by Van Bever (16) for destruction of tubercle bacilli, reduction of cream layer volumes, decrease in solubility of milk proteins, and inactivation of peroxidase and of phosphatase; and by Hetrick and Tracy (7) for inactivation of phosphatase. The data in figure 4 show that the equation for phosphatase destruction is necessarily different for different products, since different time-temperature conditions are required to inactivate phosphatase in each product. As pointed out by Van Bever, all these effects induced by heat are correlated, and, furthermore, may be attributed to heat dena-

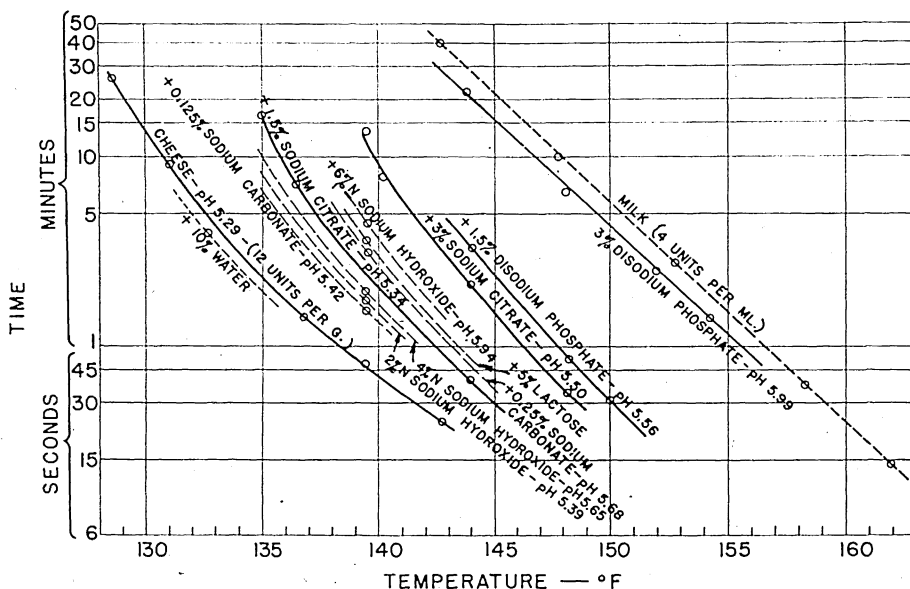


FIG. 5. Times and temperatures of heating required to reduce phosphatase activity to 3 units per 0.25 g. (12 units per g.) in cheese, and in cheese with various substances added.

turation of proteins. These relationships strengthen the belief that the phosphatase test is a reliable criterion of pasteurization.

Pasteurization holding periods required in connection with this test to reduce phosphatase activity to four units per ml. in whole milk were: 37.5 minutes at 143° F., 30 minutes at 143.7° F., 24 seconds at 160° F. and 15 seconds at 161.8° F., respectively. It will be noted that there is a slight difference between these conditions and the minimal heating conditions for pasteurization recommended in the U.S. Public Health Service milk ordinance and code. Since the straight-line semi-logarithmic graph of North and Park's (10) data, as interpreted by Dahlberg (3), shows that the Public Health Service standards allow considerably less margin of safety at 160° F. than at 143° F., some increase in the margin of safety at 160° F. seems desirable.

The temperature required to reduce the activity to four units per ml. in any given time generally was found to be about 0.7° F. lower for skim milk than for

whole milk, about 0.7°F . higher for 20 and 40 per cent cream than for whole milk, about 4.5°F . higher for ice cream mix than for whole milk and about 5.7°F . higher for sherbet than for whole milk. The time required, at 143°F ., was about three times as long for ice cream mix as for whole milk.

Results obtained in tests on Cheddar cheese and on mixtures of cheese with emulsifying salts, alkalies, lactose or water, are shown in figure 5. Phosphatase was inactivated at considerably lower temperatures and shorter holding times in Cheddar cheese than in milk, *e.g.*, to a value of 12 units per g. at 130°F . for 13 minutes and at 140°F . for slightly less than three-fourths minute in cheese having a pH of 5.29. Mixing sodium carbonate or sodium hydroxide with the cheese to increase the pH had some effect in stabilizing the enzyme against heat inactivation.

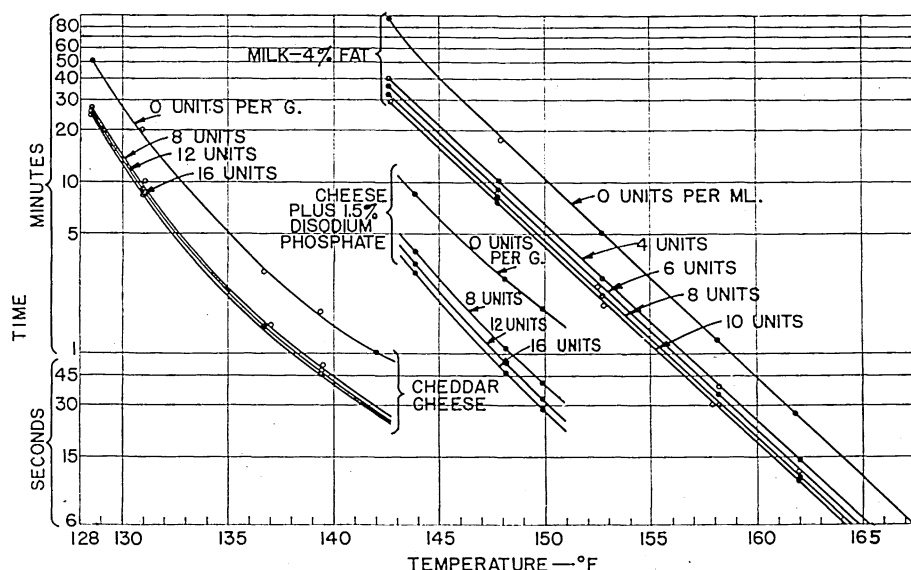


FIG. 6. Times and temperatures of heating required to cause various degrees of inactivation of phosphatase in milk, in Cheddar cheese, and in Cheddar cheese with 1.5% of anhydrous disodium phosphate added.

tion. Adding emulsifiers, such as sodium citrate or disodium phosphate, in quantities that affected the pH less, had a greater effect in stabilizing the enzyme. For example, in cheese with 1.5 per cent anhydrous disodium phosphate added (pH 5.56), a temperature of 150°F . for approximately 0.5 minute was needed to reduce the activity to 12 units per g. The addition of lactose increased the stability of the enzyme. The addition of water decreased its stability slightly.

Data showing the temperatures and times found necessary to produce various degrees of inactivation in milk and in cheese are shown in figure 6. As pointed out above, the last few remaining units were found to be the most difficult to inactivate. Differences between zero and four units were found to indicate considerably more heating than differences between four and eight units per ml. A pasteurization criterion of zero for milk, with this test, apparently would be too

severe, because it would require heating at temperatures several degrees higher than the temperatures specified in present pasteurization standards.

Figure 7 shows the effects of the pH values, at the time of heating, on the inactivation of the enzyme in milk. The heat stability was greatest when the pH was within a range of 6.5 to 7.4. In samples in which the pH was decreased progressively below 6.5, heating at 140° F. for 5 minutes decreased the activity markedly, until at pH 5.1 this heating reduced the activity to zero. There was a similar but less marked decrease in heat stability of the enzyme with increases in alkalinity beyond approximately pH 7.4.

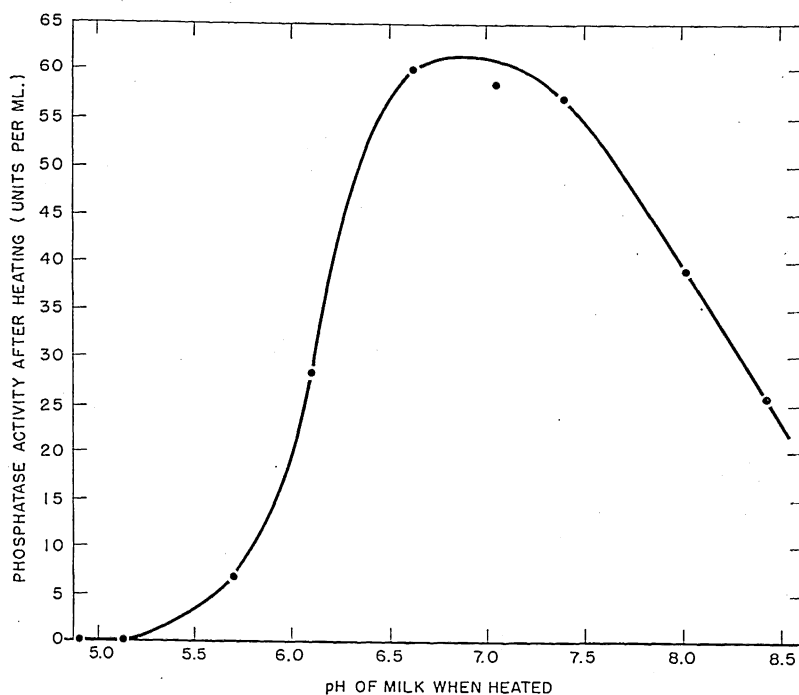


FIG. 7. Effects of pH on heat inactivation of milk phosphatase—mixture of 10% raw and 90% pasteurized milk; original phosphatase activity of mixture 195 units per ml.; heated for 5 minutes at 140° F.

DISCUSSION

Smith (14) showed that at 140° F. a considerably longer time is required to destroy tubercle bacilli in the pellicle that forms on the surface of milk than in the milk itself. Brown and Peiser (1) demonstrated that the temperature required, for a given period of time, to kill certain strains of *Streptococcus lactis* and of *E. coli* in cream is higher than that required to kill them in milk. Hening and Dahlberg (6), experimenting on the destruction of *E. coli*, inactivation of phosphatase, effect on flavor and effects on certain other properties, concluded that the present standards for pasteurization of milk are not adequate for cream. In addition to the protective effect present in cream, others (2) have shown that sugar also increases the stability of the enzyme against heat and present results

(fig. 4) corroborate this conclusion. Caulfield and Martin (2) reported that a temperature of 150° F. for 15 to 25 minutes was required to produce negative tests in ice cream mixes, and Hahn and Tracy (5) obtained similar results. It is evident that neither the present pasteurization standards for milk nor higher standards proposed by Hening and Dahlberg (6) for cream are adequate for ice cream and sherbet.

The heat stability of the enzyme is not as great in cheese as in milk. Although the stability is increased when the concentration of fat is increased (see data for cream, fig. 4), it will be noted that it is decreased much more by acidity when the pH is reduced (fig. 7) to values found normally in cheese. The acidity apparently has a predominant effect on the heat stability.

A large number of samples of process cheese, cheese foods and cheese spreads have been tested for phosphatase in these laboratories, and all of them have yielded zero values. The heating conditions used commercially in processing these products should be, and evidently are, adequate to accomplish the purposes of pasteurization.

In manufacturing cottage cheese curd from raw skim milk, a large proportion of the enzymic activity—frequently more than 80 per cent and sometimes nearly 100 per cent—is lost during manufacture. This decrease is attributed to the fact that the curd is heated, usually for a considerable period of time, after acidity has developed.

Experimental results have shown that the heat-inactivation reaction which phosphatase undergoes at the pH of normal milk is irreversible. On the other hand, when partial inactivation occurs only because of the development of acidity, the activity can be largely restored by adding sufficient alkali—*e.g.*, mixing 1 ml. of a 1.25 per cent aqueous solution of anhydrous sodium bicarbonate with 0.5 g. of cottage cheese—to increase the pH to approximately 7, and allowing the mixture to stand for several hours before testing. In the case of cottage cheese curd that has been washed thoroughly during manufacture, the presence of traces of added magnesium stimulates this reactivation, and the presence of magnesium and zinc stimulates it more.

SUMMARY

A laboratory pasteurizer is described, for controlling the heating temperatures and time accurately in pasteurization experiments.

Phosphatase test data for samples heated at any specific temperature for various periods of time show that the rate of destruction of the enzyme by heat is very rapid at first and diminishes to a relatively very slow rate with time. The experimental data for phosphatase destruction show that, in tests on milk and other fluid dairy products, a straight line results when the logarithms of the times of heating are plotted against the corresponding temperatures.

Holding periods required in this test to reduce phosphatase activity to four units per ml. of whole milk were: 37.5 minutes at 143° F., 30 minutes at 143.7° F., 24 seconds at 160° F., and 15 seconds at 161.8° F., respectively. The temperature required to produce a negative phosphatase test in any given time generally was

found to be about 0.7° F. lower for skim milk than for whole milk, about 0.7° F. higher for 20 and 40 per cent cream than for whole milk, about 4.5° F. higher for ice cream mix than for whole milk, and about 5.7° F. higher for sherbet than for whole milk. The time required, at 143° F., was about three times as long for ice cream mix as for whole milk.

Phosphatase was inactivated at considerably lower temperatures and shorter holding times in Cheddar cheese than in milk—*e.g.*, at 130° F. in 13 minutes and at 140° F. in slightly less than three-fourths minute in cheese at pH 5.29. Mixing alkalis with the cheese to increase the pH had some effect in stabilizing the enzyme against heat. Adding emulsifiers had a greater effect, as the temperature required to produce a negative test in approximately 0.5 minute was 150° F. when 1.5 per cent anhydrous disodium phosphate was added and the pH of the mixture was 5.56. The addition of lactose to cheese increased the stability of the enzyme; the addition of water decreased its stability slightly. Experiments on milks adjusted to different pH levels and heated showed that the milk phosphatase was most stable towards heat when the reaction was within a range of pH 6.5–7.4. Heating at lower or higher pH levels produced more rapid inactivation.

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THE ADAPTABILITY OF TWO STRAINS OF LACTIC STREPTOCOCCI TO GROWTH IN THE PRESENCE OF HOMOLOGOUS BACTERIOPHAGE¹

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Several investigators have reported on the adaptation of cultures of lactic streptococci to growth in the presence of bacteriophage. In all cases, the resistant cultures developed proved to be susceptible to other races of bacteriophage. In the present study, an attempt was made to determine more specifically how long it takes to adapt cultures of lactic streptococci to homologous bacteriophage, and after a strain of organisms has been made resistant, how long this characteristic will persist. Data from single trials, using two cultures of lactic streptococci and their homologous races of bacteriophage, are presented in this paper.

REVIEW OF LITERATURE

Whitehead and Hunter (5) found that a resistant culture of lactic streptococci developed by the action of bacteriophage on a sensitive strain was susceptible to attack by a new race of bacteriophage. They suggested that this type of action lends support to the theory that the bacteriophage is a product of the organism. Nelson and Hammer (3) isolated bacteriophage-resistant strains of *Streptococcus lactis* from the secondary growth of a culture upon which an inhibitory principle obtained from "slow" butter cultures had acted. Later work by Nelson *et al.* (4) showed that the secondary-growth organisms, which were not sensitive to the strain of inhibitory principle used, still were sensitive to other races of bacteriophage. Experiments by Anderson and Meanwell (1) indicate that bacteriophage-resistant strains of lactic streptococci can be developed, but on reintroduction into factory use these cultures become susceptible to secondary races of bacteriophage. Hunter and Whitehead (2) state that resistant strains of lactic streptococci usually develop sometime between 24 and 48 hours after bacteriophage has caused the lysis of a sensitive strain of organisms.

MATERIALS AND METHODS

Origin of cultures. The culture designated as H. P. is a strain of *Streptococcus cremoris* secured from the Dairy Research Institute in New Zealand. Culture no. 4 is a strain of *S. lactis* from the culture collection at Iowa State

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College. Homologous strains of bacteriophage for each were received with the cultures.

Preparation of cultures. The cultures were propagated in autoclaved re-constituted commercial dry milk solids not fat used at the rate of 90 g. per 1,000 ml. of distilled water. Transfers were made either daily or on alternate days. The cultures were incubated at 25° C. until coagulation occurred, after which they were refrigerated at 0° C. Cultures for use on any particular day were prepared by using a 1 per cent inoculum into milk late in the afternoon of the previous day.

Preparation of the bacteriophage filtrate. Sterile milk was inoculated with a milk culture of the test organism using a 1 per cent inoculum. At the same time, a few drops of whey filtrate containing the homologous bacteriophage were added. After incubation at 25° C. until coagulation occurred, usually 48 to 72 hours, the whey was filtered through a sterile Seitz filter. The resulting bacteria-free filtrate was transferred aseptically to a sterile container and stored at 0° C. until used. Several filtrates of the bacteriophage under study were prepared during the course of the experiment in order to have on hand a filtrate of maximum titer for use with each series.

Preparation of serial dilutions of bacteriophage. Serial dilutions of the whey filtrate containing bacteriophage were made directly into sterile milk. Dilutions ranging from 10^{-2} to 10^{-10} were used. The final volume, in all cases, was 100 ml. contained in a screw-capped 6-ounce prescription flask.

Titrateable acidity determination. Titratable acidities were determined on weighed 9 g. samples, using 0.1 N NaOH and phenolphthalein as the indicator. The results were expressed as per cent of lactic acid.

EXPERIMENTAL

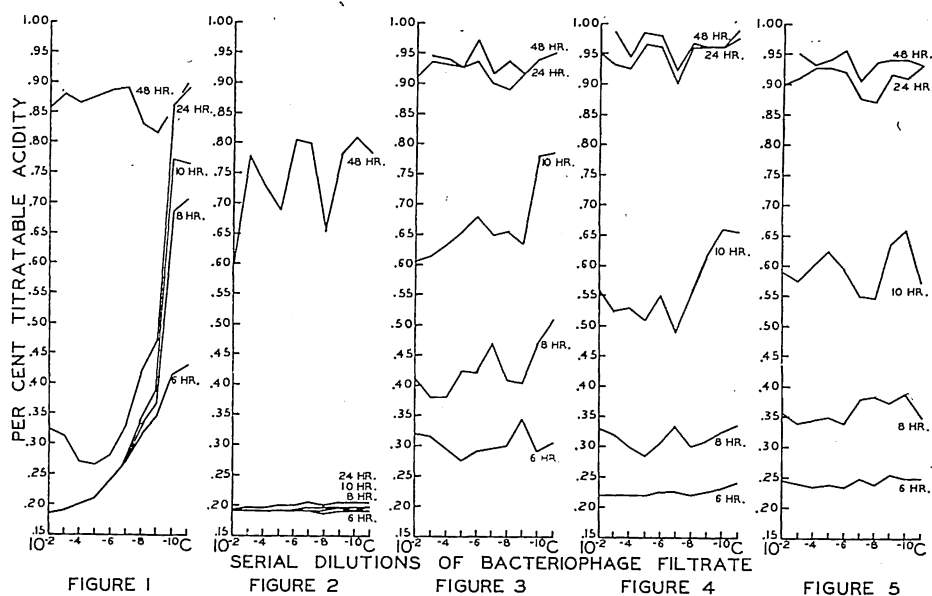
Adaptability of strain H. P. to the homologous bacteriophage. This experiment included five successive culture series. The first series consisted of ten flasks each containing 100 g. of autoclaved skim milk inoculated with a 1 g. amount of coagulated milk culture. These aliquots were dispensed aseptically from a stock flask in which the milk and the inoculum had been mixed thoroughly by vigorous agitation for a period of 1 minute. To the first nine of these flasks were added serial dilutions of the homologous bacteriophage whey filtrate ranging from 10^{-2} to 10^{-10} , inclusive. The tenth flask received no bacteriophage and served as a control to show acid production of the bacteriophage-free culture. The whole series was warmed to 30° C. in a water bath, after which the flasks were placed in an incubator adjusted to that temperature.

In the general plan of the experiment, the culture containing the lowest dilution of bacteriophage filtrate permitting coagulation of the milk after 24 hours of incubation was used to inoculate the flasks of the succeeding series. However, with series 2 it was necessary to wait 48 hours to secure a coagulated inoculum for use in the next series, as all cultures of this series, including the control, still were inhibited markedly after 24 hours by the residual bacteriophage carried over with the inoculum from series 1. Serial dilutions of the

homologous bacteriophage filtrate also were added to the flasks in all of the series by the method used in preparing series 1.

A 9 g. portion for acidity titration was withdrawn with a sterile pipette from each of the flasks after 2, 4, 6, 8, 10, 24 and 48 hours of incubation. The portions from the flasks in each series always were weighed and titrated in the same order, so as to maintain as closely as possible the desired time interval between each set of titrations.

Two series a week were started in this experiment, on Tuesdays and Saturdays, thus making one 3-day and one 4-day interval between the series of each week. The culture selected from each series for use in the succeeding series



FIGS. 1 through 5. Adaptability of strain H. P. to the homologous bacteriophage. Amount of acidity developed at 30° C.

was stored in the refrigerator at 0° C. until it was needed to make the necessary inoculation.

The data from the titrations after 6, 8, 10, 24 and 48 hours of incubation of the milk cultures are shown in figures 1 through 5. The data from the 2- and 4-hour titrations were not graphed because they did not show any significant change or differences between the various cultures containing serial dilutions of bacteriophage.

The data from series 1 (fig. 1) show that the titer of the bacteriophage filtrate used was at least as high as 10^{-9} and that the higher the serial dilution of bacteriophage filtrate added, the less was the degree of inhibition of the organisms in the culture.

The culture used to inoculate the flasks of milk of series 2 was from the 10^{-10} serial dilution of series 1. It is apparent from the data of series 2 (fig. 2) that considerable residual bacteriophage had been carried over in the inoculum from

the culture of series 1. The fact that the control culture, which had received no additional bacteriophage filtrate, was inhibited to the same extent as the others in the experimental series would seem to justify this conclusion.

The data in figure 2 also indicate that bacteriophage-resistant strains of this culture were not developed when the organisms were growing actively in the presence of a dilute concentration of bacteriophage. If any resistance to the bacteriophage had been built up in the culture used to inoculate the flasks of milk for series 2, these organisms would not have been inhibited so markedly after they had been transferred to new milk. The recovery of the organisms from the inhibiting effects of a high concentration of bacteriophage, sometime between the 24- and 48-hour titration intervals, indicates that bacteriophage-resistant strains are developed when the organisms are prevented from growing by the presence of a sufficient concentration of a homologous bacteriophage. No satisfactory explanation has been found for the rather wide and inconsistent variations in the titratable acidities after 48 hours of incubation of the cultures in series 2.

The data from series 3 (fig. 3) show that strains with some degree of bacteriophage resistance were developed sometime near the end of series 2, because the rate of acid production of nearly all cultures containing added bacteriophage was much more rapid at the 6-, 8- and 10-hour titration intervals in series 3 than was the case in either series 1 or 2.

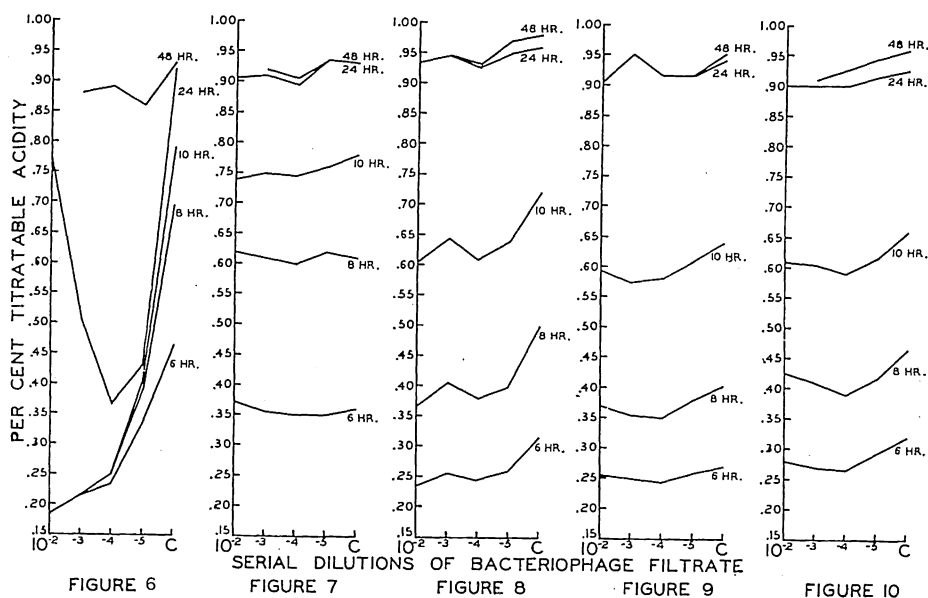
The steadily increasing resistance to bacteriophage of the newly developed strains of lactic acid organism became noticeable soon after the start of series 3. In series 3 and 4 (figs. 3 and 4) the culture which received the greatest concentrations of added bacteriophage filtrate still showed some inhibition in acid production as compared to the controls, especially at the 10-hour titration period, but in series 5 (fig. 5) the acidities followed very nearly a straight line across the graph, except for slight variations which could well be expected. The fact that the culture of series 5, which contained a serial dilution of 10^{-2} of bacteriophage filtrate, showed practically the same titratable acidity at each titration interval as the control indicates that the lactic streptococci of this strain had become well adapted to growth in the presence of active bacteriophage after only a relatively few transfers in milk to which bacteriophage filtrate had been added.

The somewhat lower acidities developed by each culture in series 3, 4 and 5 at the 6-, 8- and 10-hour titration periods as compared to the control of series 1, which was inoculated from an actively growing culture, can be attributed, in part at least, to the general "slowing down" effect on organisms in cultures that are not transferred frequently enough. The magnitude of this effect could have been determined in the present experiment had a control culture, with no added bacteriophage filtrate, been included with each experimental series. However, at the 24- and 48-hour titration intervals the titratable acidity of practically every culture in series 3, 4 and 5 was higher than the control culture of series 1.

Adaptability of strain no. 4 to the homologous bacteriophage. This experiment included five successive culture series. The general experimental procedure

was the same as that used in the experiments with strain H. P. In the studies with the no. 4 strain, only five flasks were used in each series because the titer of the bacteriophage filtrate active against this organism, as shown by preliminary observations, was not very high. To four of these flasks were added serial dilutions of the homologous bacteriophage filtrate ranging from 10^{-2} to 10^{-5} , inclusive, and the fifth served as a control.

The data from the titrations after 6, 8, 10, 24 and 48 hours of incubation of the milk cultures are presented in figures 6 through 10. The data from the 2- and 4-hour titrations were not graphed because they did not show any significant change in acidity.



Figs. 6 through 10. Adaptability of strain no. 4 to the homologous bacteriophage. Amount of acidity developed at 30°C .

The data from series 1 (fig. 6) indicate that the titer of the bacteriophage filtrate used in this experiment was at least as high as 10^{-5} . It cannot be stated conclusively whether or not it was higher than this, because a culture containing a serial dilution of 10^{-6} of filtrate was not included in this experimental series. It is very evident, however, that the bacteriophage filtrate active against culture no. 4 was not nearly as potent as the one used in the studies with culture H. P. It will be noted further that the organisms in two of the cultures in series 1 had become fairly well adapted, sometime between the 10- and 24-hour titration periods, to growing in the presence of the bacteriophage to which they were initially susceptible. The titratable acidity readings of series 1 at the 24-hour interval are very significant, because they show that the organisms of this particular culture developed a resistance to the bacteriophage more quickly in the presence of a heavy inoculation of the bacteriophage filtrate than they did when

a lesser quantity was added. The earlier recovery was particularly noticeable in the cultures to which serial dilutions of 10^{-2} and 10^{-3} of bacteriophage filtrate were added.

The results show that culture no. 4 had attained its maximum adaptability for growth in the presence of the homologous bacteriophage by the end of series 2 (fig. 7). The somewhat lower acidities developed by each culture in series 3, 4 and 5 (figs. 8, 9 and 10) at the 6-, 8- and 10-hour titration periods as compared to the control of series 1 and all cultures in series 2 can be attributed, at least partially, to the general "slowing down" effect on organisms in cultures that are not transferred frequently enough. It will be noted, however, that there were no great differences in the titratable acidities of any of the cultures in series 2, 3, 4 and 5 at the 24- or 48-hour titration intervals.

TABLE 1
Comparison of adapted and non-adapted cultures after storage at 0° C.

Hr. incubated at 30° C.	Titratable acidity (% lactic acid)					
	Culture—H. P.			Culture—no. 4		
	Adapted	Non-adapted	Control	Adapted	Non-adapted	Control
After 1 month						
2	0.190	0.185	0.180	0.180	0.185	0.180
4	0.255	0.185	0.220	0.240	0.220	0.225
6	0.390	0.185	0.330	0.400	0.235	0.345
8	0.585	0.185	0.535	0.620	0.235	0.555
10	0.705	0.185	0.670	0.725	0.250	0.695
24	0.820	0.375	0.800	0.815	0.445	0.825
48	0.820	0.730	0.805	0.830	0.830	0.860
After 4.5 months						
2	0.175	0.180	0.180	0.180	0.180	0.180
4	0.195	0.195	0.230	0.245	0.250	0.250
6	0.195	0.195	0.345	0.410	0.370	0.420
8	0.195	0.195	0.600	0.590	0.390	0.590
10	0.200	0.195	0.685	0.685	0.405	0.685
24	0.520	0.250	0.740	0.795	0.430	0.810
48	0.760	0.720	0.760	0.815	0.610	0.830

The ability of adapted strains of organisms to retain their adaptability to a specific race of bacteriophage. Transfers were made of the adapted cultures at intervals of 10 to 14 days. Except for the time during which newly-inoculated cultures were being incubated, the cultures were stored in the refrigerator at 0° C. Approximately one month after the completion of the original adaptation studies, each of the "adapted" cultures was compared with a normal culture of the same strain to determine if the acquired characteristic was temporary, or whether it persisted after repeated transfers to new milk. The procedure for this test was as follows: One milliliter of the adapted culture was added to a flask containing 100 ml. of autoclaved milk. Also, 1 ml. of a normal culture of the same strain of organism was added to each of two similar flasks of milk. One milliliter of bacteriophage filtrate active against the strain of or-

ganism then was added to the flask containing the adapted inoculum, and the same amount of filtrate was added to one of the other flasks. The third flask of milk served as a control. The cultures were incubated at 30° C. Titratable acidity determinations were made after 2, 4, 6, 8, 10, 24 and 48 hours of incubation. The results of these determinations are presented in table 1. The data indicate that both the adapted H. P. culture and the adapted no. 4 culture retained their ability to resist attack by the bacteriophage active against the original cultures of the same strains of organisms. The same type of determination was made on these cultures after 4.5 months of storage at 0° C. The results of this experiment also are presented in table 1. The results of this trial

TABLE 2
*Comparison of adapted and non-adapted cultures after approximately
4.5 months of storage at 0° C.*

Hr. incubated at 30° C	Titratable acidity (% lactic acid)					
	Culture—H. P.			Culture—no. 4		
	Adapted	Non-adapted	Control	Adapted	Non-adapted	Control
2	0.175	0.180	0.180	0.180	0.180	0.180
4	0.195	0.195	0.230	0.245	0.250	0.250
6	0.195	0.195	0.345	0.410	0.370	0.420
8	0.195	0.195	0.600	0.590	0.390	0.590
10	0.200	0.195	0.685	0.685	0.405	0.685
24	0.520	0.250	0.740	0.795	0.430	0.810
48	0.760	0.720	0.760	0.815	0.610	0.830

show that the adapted H. P. culture had almost completely lost its previously-acquired resistance to the homologous bacteriophage. The only indication that it had retained a part of its acquired resistance was the fact that the titratable acidity of the adapted culture was markedly above the acidity of the normal culture at the 24-hour titration interval. The adapted no. 4 culture developed acid just as rapidly as the control culture, which contained no added bacteriophage; therefore, it must be concluded that it had retained its acquired resistance to the bacteriophage active against it even after 4.5 months.

These data indicate that one cannot predict accurately how long adapted cultures of lactic acid streptococci will retain the resistance that they acquire when grown in the presence of a homologous bacteriophage. The limited data available show that the degree of retention of this acquired characteristic will vary between different strains of organisms.

SUMMARY

In the present study, experiments were conducted to determine if single-strain cultures of lactic-acid streptococci could become adapted to grow in the presence of homologous bacteriophage, and if so, how long the acquired characteristic would persist.

The data show that single-strain cultures of lactic streptococci will acquire

an adaptation for growth in the presence of homologous bacteriophage. The length of time during which this acquired characteristic will persist varies with the strain of organism.

ACKNOWLEDGMENTS

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EFFECT OF HIGH-TEMPERATURE SHORT-TIME HEAT TREATMENTS ON SOME PROPERTIES OF MILK. I. INACTIVATION OF THE PHOSPHATASE ENZYME¹

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Since the development of the phosphatase test by Kay and Graham (8) and its subsequent modifications, several studies have been made to determine the suitability of the test for detecting irregularities in the pasteurization of milk and other dairy products. The literature on this phase of the phosphatase test up to 1939 has been thoroughly reviewed by Burgwald (2). The reported studies showed that all raw milk contains phosphatase, that the thermal resistance of phosphatase is greater than that of pathogens, and that the test is sufficiently sensitive to detect important variations in pasteurizing conditions on the basis of the residual phosphatase activity. Other work has been directed toward improvement of the phosphatase test to make it more sensitive, more rapid, and more quantitative (3, 5, 13, 15).

Several investigators (6, 7, 11, 14, 17) have reported on the time-temperature relationships necessary to inactivate the phosphatase enzyme in milk. The results obtained differ considerably, probably because of the variety of methods used for heating the milk and for testing the phosphatase activity, as well as differences in accepted standards of what constitutes satisfactory destruction of the enzyme. Holland and Dahlberg (6) stated that most of the discrepancies probably could be accounted for by the variations in the length of time required to heat to and cool from the temperatures at which they are holding. They did not, however, present any data to show the effect of various rates of heating. Later Lythgoe (9) commented that if milk is heated very quickly the time of inactivation of phosphatase necessarily may be longer than if milk is heated more slowly, but no data were given.

Previous experimental work has shown that at temperatures above 140° F. phosphatase destruction proceeds with sufficient rapidity to make time of heating to temperature extremely important and, as the temperature to which milk is heated becomes higher, the cumulative effects of heat in reaching the temperature become progressively more important. Using a heating time of 35–40 seconds, Prucha and Corbett (11) found the phosphatase to be inactivated by an instantaneous exposure at 160° F. They used Scharer's (15) test, measuring the indophenol color with the Hahn and Tracy (5) photoelectric cell set-up, and placed the standard for satisfactory destruction of phosphatase at 0.8 p.p.m. phenol equivalent. Holland and Dahlberg (6) secured a negative phosphatase

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test by heating to 170° F. using a 10-second heating time above 140° F. and the Kay and Graham (8) test with the standard of 0.04 mg. phenol equivalent.

In June, 1946, an experimental unit built on Mallory (10) principles by the Illinois Creamery Supply Company, Chicago, Illinois, was installed in the research laboratory of the Dean Milk Company, Rockford, Illinois. With this equipment, it was possible to heat cold milk to any desired temperature well above the boiling point in 0.83 second.

Since the trend in design of continuous milk heating equipment is toward faster, more efficient heating with more precise control of flow rate and temperature, it would seem that higher temperatures and shorter time processing will result and more information will be necessary to set time-temperature relationships. It also was apparent that information should be secured on the effects of various rates of heating on the temperatures required to inactivate the phosphatase to provide a better basis for evaluating the results of other investigators in this field. The results of such a study could be used to form a basis for formulating a practical mathematical time-temperature solution for the inactivation of the phosphatase enzyme which would explain the results secured by any heating method. It also was decided to study seasonal variations in phosphatase content of milk and the distribution of the phosphatase enzyme in various milk fractions.

EXPERIMENTAL METHODS

All of the experimental work was done in the research laboratory of the Dean Milk Company. The milk used for this study was fresh Grade A raw milk as received at the plant from the company's patrons. The Mallory small-tube heat exchanger was used to process the milk when various times and temperatures required to inactivate the phosphatase enzyme were studied. This unit had a capacity of 80 gallons per hour. It was composed of five independently operating heating sections and two cooling sections. Milk was forced through the unit with an 80-gallon-per-hour Manton-Gaulin homogenizer used as a high pressure pump. A pressure of 800 to 1,000 lb. per square inch was required to force milk through the unit. Each heating section was composed of four 58.5-inch lengths of $\frac{1}{4}$ -inch O. D. stainless steel tubing, through which the milk flowed, surrounded by a larger pipe containing dry steam as the heating medium. The time required for the milk to flow through one heating or cooling section was calculated to be 0.83 second and the milk flowed through the unit at a calculated velocity of 23.6 feet per second. After the milk was heated to the desired temperature, it flowed through a copper coil of $\frac{3}{8}$ -inch O. D. immersed in a water bath held at the desired holding temperature. The coil was so constructed that milk could be removed after any desired length of time directly into test tubes which previously had been immersed in ice water. The temperatures were measured with a mercury-in-glass thermometer inserted in a mercury well which was placed in the line between the Mallory unit and the copper holding coil. In this study, the time required to heat to all maximum temperatures was 0.83 second.

The phosphatase activity in terms of phenol equivalent was measured by the

method of Sanders and Sager (13) with one modification; *i.e.*, the color development buffer used was the one proposed by them (12) in a previous publication. One-hour incubation time at 37° C. and half-hour color development at room temperature were used. The indophenol was extracted with 10 ml. of buffered butyl alcohol and the transmission measured at 650 m μ , using a Coleman model 11 spectrophotometer and a 1.3-cm. square cuvette. Boiled milk controls were run with each series of determinations. The quantities of phenol, after consideration of the boiled controls, were read directly from a standard transmission-concentration curve prepared with known amounts of phenol. The results are expressed as micrograms of phenol per milliliter of milk or parts per million on a milk basis (not on the basis of the parts per million phenol in the butyl alcohol extract). This method was found to be sensitive and reproducible quantitatively; 0.05 per cent raw milk in boiled milk could be detected readily. Phenol added to pasteurized milk could be recovered satisfactorily.

The phosphatase tests were run approximately 24 hours after treatment of the milk.

RESULTS

Phosphatase activity in raw milk and distribution of the phosphatase enzyme in various milk fractions

Variations in the initial phosphatase concentration, if large, possibly would make some difference in the time and temperature required to inactivate the enzyme. Unfortunately, the phosphatase contents of the raw milk in the initial stages of the study were not accurately determined and only those values determined since February, 1947, will be given. The initial phosphatase content was so high that insufficient di-sodium phenyl phosphate was used and this was not recognized until the values were checked by diluting the raw milk samples with boiled milk to bring the phenol concentrations within the range of the standard curve concentration. Since the kinetics of the reaction between the enzyme and the substrate in the test itself is reported to be first order, the initial concentration will have some effect on the rate of the reaction when a standard length of time of incubation is used. For this reason all raw samples were diluted with boiled milk to make the final phenol concentration fall within a rather narrow range (range of standard curve, 0–20 γ phenol), and the concentrations then were calculated on the raw milk basis.

The phosphatase values of ten lots of raw milk from February 20 to July 9, 1947, ranged from 1,920–3,000 p.p.m. with an average of 2,230 p.p.m. These values are of the same order of magnitude as the value reported by Sanders and Sager (13) for whole milk and are considerably higher than those secured by previous investigators.

To secure some idea of the distribution of phosphatase in milk, raw milk was separated into cream and skim milk. The raw cream was churned into butter and the raw buttermilk drained off. The butter was melted at 110° F. and centrifuged to secure butter oil devoid of phospholipid material. The pH of the raw skim milk was adjusted to pH 4.6 with 0.1 N hydrochloric acid and the casein

was filtered off. The filtrate was neutralized to pH 6.7 with 0.1 *N* sodium hydroxide and the phosphatase activities of all fractions were determined. Each sample was diluted with boiled milk before running the phosphatase test to bring the final phenol concentrations in range of the standard curve. The results are calculated on the product basis by weight and reported as p.p.m. phenol equivalent. The results (fig. 1) indicate that the enzyme probably is concentrated at the fat-serum interface, perhaps in a manner similar to agglutinin, since butter oil showed no phosphatase activity and the buttermilk showed an activity approximately ten times that of skim milk. This is in agreement with the observations of Kay and Graham (7). It should be observed, too, that the phosphatase activity of the raw milk was recovered quantitatively in the skim milk and cream.

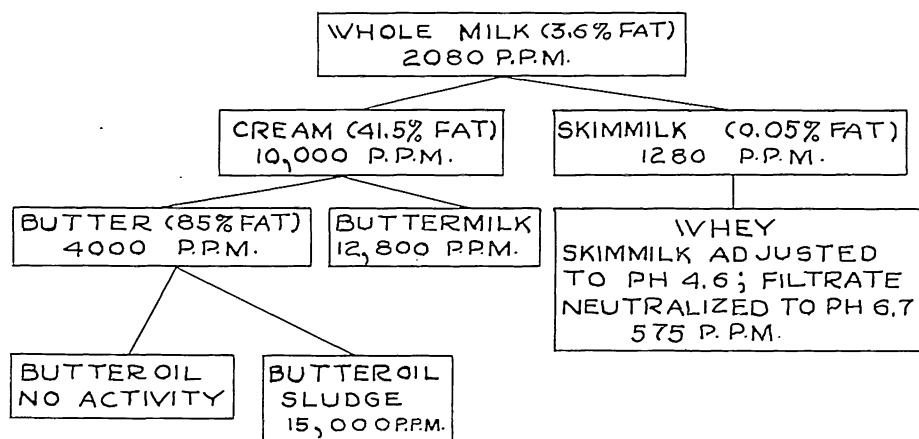


FIG. 1. Distribution of phosphatase in the various milk fractions. Phenol equivalent on the product basis by weight (p.p.m.).

The separation temperature in this case was 86° F. Figure 1 also shows that there is sufficient phosphatase activity in the various milk fractions to permit use of the phosphatase test to determine whether or not most dairy products have been properly pasteurized. The only exception appears to be butter oil.

Since the phosphatase enzyme apparently was concentrated at the fat-serum interface, a study of the effect of separating temperature on the distribution of the enzyme between cream and skim milk was thought desirable. If held in a similar manner as agglutinin, it was thought that an increase in the separating temperature would result in release of the enzyme from fat and the skim milk upon separation would show more enzyme activity. Consequently three lots of raw milk from the same milk source were separated, one at 80° F., another at 100° F., and a third at 120° F. Each lot of cream was standardized to 39.5 ± 0.5 per cent fat with skim milk from the same separation, and all samples (milk, cream and skim milk) were tested for phosphatase activity. The results are recorded in table 1.

Although some reduction in phosphatase activity of the cream resulted from increasing the separating temperature, a corresponding increase in the skim milk

fraction was not observed, which suggests the possibility that some phosphatase activity may be lost in the separator slime as the separating temperature is increased. It commonly is observed that as the temperature of separation and centrifugal clarification increases, the quantity of slime also increases, which may account for the losses in phosphatase activity observed in the cream (table 1). Actual test of the separator slime in another experiment showed it to contain considerable phosphatase activity. The reduced phosphatase activity also may be due to slight inactivation by the higher temperatures, but this is not borne out by data to be presented later. At any rate, the phosphatase apparently is held to fat more tenaciously than is agglutinin. It should not be inferred, however, that the phosphatase is entirely on the fat.

TABLE 1

Effect of separating temperature on the distribution of phosphatase in the cream and skim milk fractions

Separating temp. (°F.)	Phosphatase activity (p.p.m. phenol)			
	Milk	Cream	Skim milk	Reconstituted milk
80	2,130	10,400	1,370	2,190
100	2,100	9,400	1,360	2,090
120	2,090	8,600	1,340	2,000

Selection of phenol concentration standard for satisfactory inactivation of the phosphatase enzyme

The distribution of phosphatase activity in cream and skim milk brought up an interesting thought with respect to phenol standards for satisfactory destruction of the enzyme. If one were to pasteurize milk and select the 4 p.p.m. phenol equivalent standard as proposed by Sanders and Sager (13), cream separated from this milk would, it was thought, show a positive phosphatase test. Positive phosphatase tests on cream separated from pasteurized milk have been reported by Scharer (16), using his rapid method.

Milk was heated in the steam jacketed hot well in such a way as to give phenol equivalents greater than 4 p.p.m. and less than 4 p.p.m. and the resulting milk cooled and held two hours. The lots of milk then were heated to 80° F. and separated into 32 per cent cream and skim milk, and the phosphatase activities of the milk, cream, and skim were determined to see if the distribution of the enzyme would be essentially the same as when unheated milk was separated. When milk with a phosphatase activity of 7.5 p.p.m. was separated, the cream and skim milk showed phosphatase activities of 12.8 and 5.4 p.p.m., respectively. When milk with a phosphatase activity of 3.3 p.p.m. was separated, the phosphatase of the cream and skim milk separated from this milk were found to be 4.9 and 2.2 p.p.m., respectively.

While the cream showed much higher phosphatase activity than the milk, the differences were less than when cream and skim milk were separated from raw milk. The cream showed a positive phosphatase test even when the milk was

properly pasteurized according to the Sanders and Sager (13) standard. In another experiment in which milk was heated to give a phosphatase activity of 1.9 p.p.m. phenol equivalent and the resulting milk separated into 25 per cent cream, the cream showed a phosphatase activity of 2.8 p.p.m. phenol. Apparently this relationship would hold no matter what phenol concentration is selected unless the phosphatase were completely inactivated. However, the lower the phosphatase activity of the milk the smaller the spread between the phosphatase activity of milk and cream seems to be. Then too, values of less than 1.0 p.p.m. are not readily distinguishable by the test. To minimize the discrepancy between properly pasteurized milk and cream separated therefrom, keeping in mind the accuracy of the test, a value of 1.0 p.p.m. was adopted as the standard for satisfactory inactivation in this study. This standard requires temperatures higher, approximately 1–3° F., depending upon the corresponding holding time, than the 4 p.p.m. standard of Sanders and Sager (13). Gilcreas and O'Brien (4) indicated that higher temperatures than present pasteurization standards are necessary to kill *E. coli*, which may justify elevating the standards for the phosphatase test.

From a practical standpoint, it should be pointed out as a conclusion to this phase of the study that either the phenol standards must be varied to suit the various dairy products being pasteurized at constant time-temperature conditions or, if a single phenol standard is selected, more severe heat treatments will have to be administered to some products than others to call them properly pasteurized. The data of Sanders and Sager (14) verify this conclusion.

Kinetics of inactivation of phosphatase by heat treatment and time-temperature relationships

If the reaction is first order, as indicated by Van Bever and Straub (17), a plot of the log of the concentration against the time should yield a straight line. Milk was heated in the hot well to 143° F. and the relationship of time at constant temperature (143° F.) to the concentration is given in figure 2 for skim milk and whole milk. The reaction is not strictly first order but possibly a pseudo-first order. Much the same results were secured at higher temperatures using the Mallory unit to heat and the holding accomplished with the coil described previously. These data are given in table 2.

Van Bever and Straub (17) have derived a mathematical expression from the first order reaction rate formula and Arrhenius equation with which they should be able to predict the residual phenol concentration for any time-temperature cycle if these relationships hold. From the results shown in figure 2, this did not seem to be readily possible because the reaction did not appear to be strictly first order. As the destruction of phosphatase proceeded, the rate of destruction at constant temperature became slower with time than would be indicated by straight semi-log relationship. This difference possibly could be explained by differences in the method of determination of the phosphatase used in this investigation and that used by Van Bever and Straub (17), and differences in the range

of concentrations studied. They found the relationship to hold during up to 96 per cent destruction of the original phosphatase content.

The thermal processing work of Ball (1) suggested an approach to the problem through mathematical solution. Several investigators (6, 8, 14, 17) have

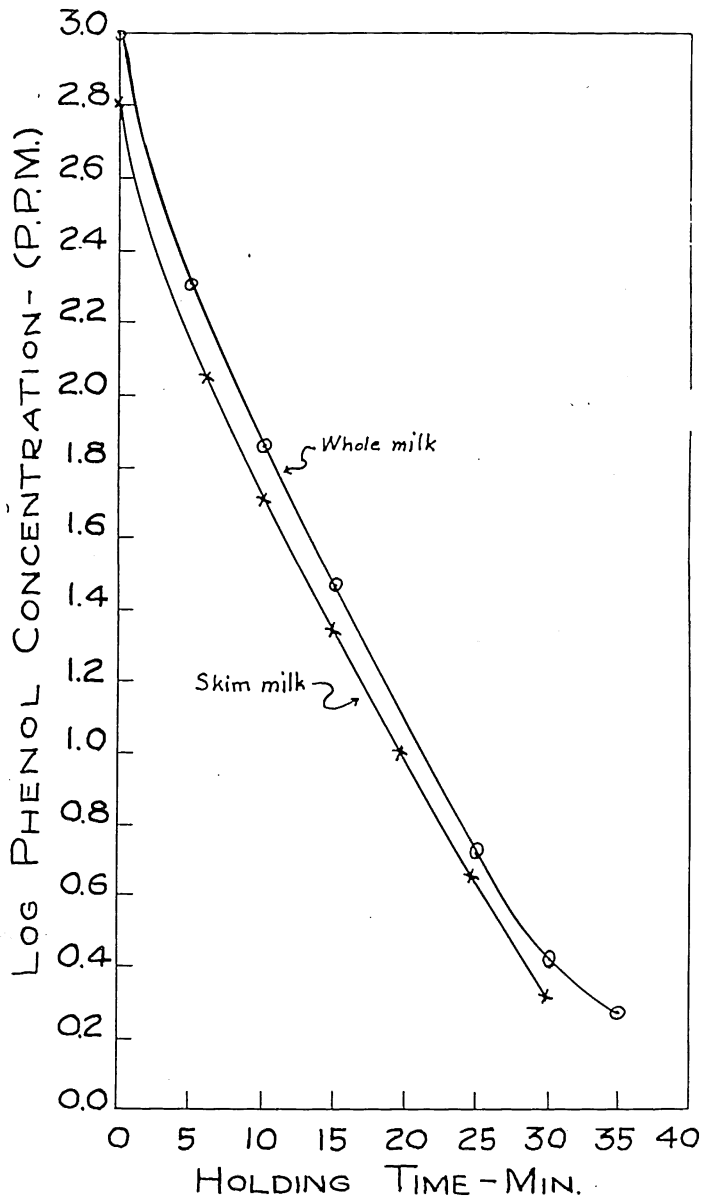


FIG. 2. Change in phosphatase concentration with time at 143° F.

shown that a plot of the temperature vs. the log time for inactivation at that temperature yields a straight line. Using 0.8-second heating time and holding in the coil described previously, the times required at various temperatures from 143 to

185° F. to reduce the phosphatase concentration to 1 p.p.m. phenol equivalent were determined. The results plotted in figure 3 show the same relationship secured by previous investigators. The formula for the curve shown in figure 3 is: $T = 174 - 9 \log t$, where T is the temperature in °F. and t is the time in seconds required at temperature T to inactivate the phosphatase. From this relationship it would seem that a practical solution for time-temperature cycles could

TABLE 2

*Effect of time and temperature on the phosphatase activity in whole milk
(0.8 sec. required to heat to all temperatures)*

Temperature	Time	Phenol
(°F.)	(sec.)	(p.p.m.)
Raw milk	2,180
150	306	9.3
	361	2.0
	420	1.0
155	2	1,265
	32	67
	64	15
	92	1.6
	112	0.9
Raw milk	2,020
160	2	340
	13	6.0
	22	1.4
	32	0.6
165	2	55
	7
	10	1.0
	15	0.6
Raw milk	3,000
159	.03	2,480
165	.03	1,580
170	.03	855
175	.03	137
180	.03	4.8
185	.03	1.0
171	1	11.8
172	1	6.0
173	1	1.6
174	1	1.0
175	1	0.4

be developed if one considered a single final phenol concentration (1 p.p.m.). The destructive effect (hereafter called the D value) of any temperature for each second hold in inactivation of the phosphatase (1 p.p.m.) can be given as: $D = \log^{-1}_{10} \frac{(T - 174)}{9}$ and the summation of the D values times the time at the corresponding temperatures must be 1 or greater to insure satisfactory inactivation of the phosphatase.

Using this formula it can be shown that it would require 6 hours to inactivate the enzyme at 135° F. This was verified experimentally and this temperature was selected as a starting point for calculating the D values listed in table 3. The third column in table 3 shows the cumulative D values at any temperature as-

suming straight line heating and 1-second per degree F. heating rate. It should be stated that this was arranged for convenience in calculating cumulative D values for experimental data secured later using constant heating rates. But, according to this method, one could calculate a cumulative D value by plotting the individual D values corresponding to the temperatures used against the time in seconds. The area under the resulting curve would be the cumulative D value and this should be 1 or greater for any time-temperature cycle if the phosphatase is inactivated.

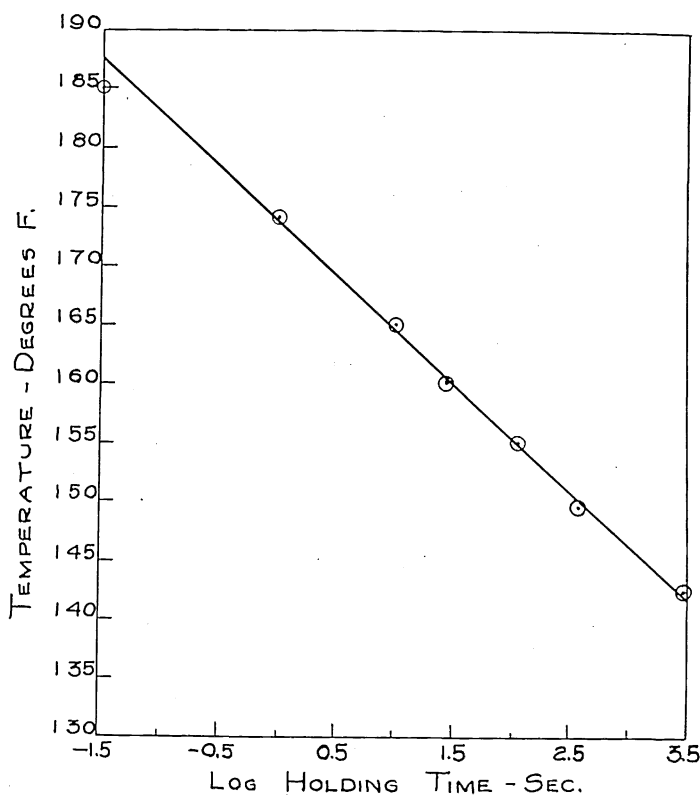


FIG. 3. Time-temperature relationship for the inactivation of the phosphatase enzyme in milk.

Effect of heating rate on temperature necessary to inactivate the phosphatase enzyme in milk

To secure some evidence on whether or not the mathematical solution has practical and proved application and to find the relationship between hot well heating at various rates and Mallory heating, the following experiments were performed.

Raw whole milk was heated in the hot wells by varying the quantities of milk and steam so that various line (constant change in temperature with time) heating and various heating rates from 2 to 20° F. per minute were secured. Samples were obtained at various temperatures and cooled rapidly in ice water as the

milk was being heated; the temperature at which the phenol concentration became 1 p.p.m. was determined. The results are listed in table 4, along with

TABLE 3
Comparative destructive effect of heat on the phosphatase enzyme activity in milk

Temperature (°F.)	D value/sec. hold	Cumulative D value (1 sec./°F.) (Straight line heating)
135	0.000046	0.000046
136	0.000059	0.000105
137	0.000077	0.000182
138	0.0001000	0.000282
139	0.000129	0.000411
140	0.000167	0.000578
141	0.000215	0.000793
142	0.000277	0.001070
143	0.000358	0.001428
144	0.000462	0.001890
145	0.000597	0.002487
146	0.000772	0.003259
147	0.00100	0.00426
148	0.00129	0.00555
149	0.00167	0.00722
150	0.00215	0.00937
151	0.00277	0.01214
152	0.00358	0.01572
153	0.00462	0.02034
154	0.00597	0.02631
155	0.00772	0.03403
156	0.0100	0.0441
157	0.0129	0.0570
158	0.0167	0.0737
159	0.0215	0.0952
160	0.0277	0.1229
161	0.0358	0.1587
162	0.0462	0.2049
163	0.0597	0.2646
164	0.0772	0.3418
165	0.100	0.442
166	0.129	0.571
167	0.167	0.738
168	0.215	0.953
169	0.277	1.230
170	0.358	1.588
171	0.462	2.050
172	0.597	2.647
173	0.772	3.419
174	1.00	4.42
175	1.29	5.71
176	1.67	7.38
177	2.15	9.53
178	2.77	12.30
179	3.58	15.88
180	4.62	20.50
181	5.97	26.47
182	7.72	34.19
183	10.0	44.2
184	12.9	57.1
185	16.7	73.8

data secured by Mallory heating and holding. The corresponding calculated cumulative D values also are shown. The dates on which the individual experi-

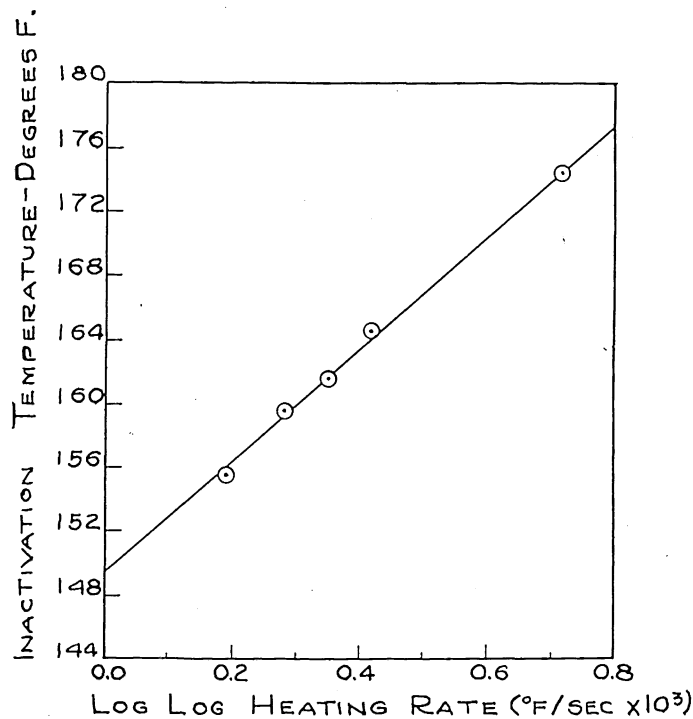


FIG. 4. Effect of heating rate on the temperature required to inactivate the phosphatase enzyme in milk.

ments were performed are given to indicate any effect season may have had on time-temperature relationships.

As a sample calculation of the cumulative D values, consider the first set of values given in table 4. The heating rate was 0.035° F. per second or the milk was held 28.6 seconds per $^{\circ}$ F. as it was heated from 135 to 155° F. The cumulative D value at 155° F. is 0.03403 if held only 1 second per $^{\circ}$ F. Therefore, by

TABLE 4

Effect of heating rate on the temperatures required to inactivate the phosphatase enzyme in milk (1.0 p.p.m. phenol equivalent)

Type of heating	Date	Heating rate ($^{\circ}$ F./sec.)	Temp. ($^{\circ}$ F.)	Holding time (sec.)	D value
Hot well	10/ 1/46	0.035	155	1	0.98
	10/21/46	0.085	159	1	1.14
	9/17/46	0.167	161	1	0.99
	9/17/46	0.390	164	1	0.95
	10/ 1/46	0.178	143	3,000	1.08
	11/27/46	0.177	152	270	1.05
Mallory	8/ 9/46	137	174	1	1.03
	3/28/47	150	185	0.03	0.99
	5/14/47	120	165	10	1.00
	5/14/47	108	150	420	0.91

the time the milk reached 155° F., the D value equals 28.6×0.03403 or 0.97. To this must be added the D value \times time at 155° F. = $0.0077 \times 1 = 0.0077$. Thus, the cumulative D value would be 0.98.

Figure 4 shows the relationship between heating rate and temperature necessary to inactivate phosphatase with one second hold at the top temperature. The temperature of inactivation is a log log function of the heating rate. Extending the curve to 0 log log heating rate ($^{\circ}\text{F.}/\text{sec.} \times 10^3$) indicates a temperature of 149° F. to be sufficient to inactivate the phosphatase. Theoretical calculations from the D values indicate a temperature of 150° F., which is fairly good agreement.

Apparently the mathematical solution is essentially satisfactory considering the constancy of the cumulative D values. If one considers an error of 1° F. in top temperature as being possible, the cumulative D values may vary approximately 0.2 and all values listed in table 4 are within this limit.

SUMMARY

Information was secured on the phosphatase activity of raw milk and the distribution of phosphatase in various milk fractions. The phosphatase is believed to be located to a large extent but not entirely at the fat-serum interface. Separation temperatures up to 120° F. did not appreciably change the distribution of the phosphatase in the cream and skim milk fractions. Separation of heated milks possessing various phosphatase activities showed that the lower the phosphatase activity in the milk the less the difference in the phosphatase activity in the cream over that in the milk. A standard of 1 p.p.m. phenol was selected as satisfactory destruction considering the limits of accuracy of the test and the smallest increase from a practical standpoint in phosphatase activity in cream over that in milk from which this cream was separated.

A study was made of the kinetics of phosphatase inactivation by heat, and time-temperature relationships for the inactivation of phosphatase are given over the temperature range of 143 to 185° F.

A mathematical solution for time-temperature cycles is given which takes into consideration accumulative effects of heating to the holding temperature in reducing the phosphatase activity to 1 p.p.m. phenol. Data secured with various rates of heating indicated that the mathematical solution is satisfactory for practical use in determining time and temperature necessary to give a negative phosphatase test in milk with various heating methods. It follows that, if the phosphatase test is used as the standard for adequate pasteurization, this mathematical solution can be applied to determine the proper time-temperature conditions.

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EFFECT OF HIGH-TEMPERATURE SHORT-TIME HEAT TREATMENTS ON SOME PROPERTIES OF MILK. II. INACTIVATION OF THE LIPASE ENZYME¹

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Although there was considerable disagreement in the early literature concerning the presence of lipase enzyme in milk, the work of Rice and Markley (13) and Palmer (11) in 1922 showed lipase to be a normal constituent of cow's milk.

This enzyme is capable of splitting the glyceride structure of milk fat, liberating free fatty acids. The low molecular weight fatty acids thus formed, such as butyric and caproic acids, give the milk its characteristic rancid odor and flavor. It appears as though there may be more than one lipase present in milk (6). In general, two types of lipolysis now are recognized; one is the spontaneous and the other induced lipolysis. This study deals with the induced type of lipolysis. In induced lipolysis, the system must be "activated", possibly by changing the normal adsorbed layer surrounding the fat globules, thus making the fat more susceptible to lipase action. This can be accomplished by shaking (9), by homogenization at temperatures below 130° F. (2), and by temperature treatment between 40–80° F. (8). In this study, lipolysis was induced by homogenization at 105° F.

The lipase enzyme is heat labile. While it is not known whether both types of lipolysis respond similarly to heat treatment, the indications are that both types are destroyed under standard pasteurization conditions using the holder method. Tarassuk (14) found that 130° F. for 30 minutes would prevent the spontaneous type of lipolysis. Dorner and Widmer (2) and Halloran and Trout (5) found that pasteurization before homogenization stopped this type of induced lipolysis. Doan and Minster (1) have shown that rancidity invariably was present 24 hours after homogenization unless the milk had been previously heated to 150° F. without holding. Gould and Trout (4) found no change in fat constants or acid degree of milk fat when milk was pasteurized at 145° F. for 30 minutes and homogenized at a pressure of 1,500 lb. per square inch. In their experiments, homogenization of raw milk caused an average increase of 1.625 per cent in fat acidity during the first 24 hours of storage at 35–40° F. In an experiment where milk was heated to 140, 150, 160 and 170° F. without holding and the milk subsequently made into sweetened condensed milk, Rice (12) found that only the milk preheated to 140° F. developed rancid flavor after 8 months of storage at room temperature. When milk was heated in the

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presence of sugar, a temperature of 180° F. was necessary to prevent this defect. Palmer (11) stated that heating milk to 75° C. (167° F.) for a few minutes would stop rancid and bitter flavor development. In these studies the heating times were not given.

The present study was designed to secure more information on the holding time of temperatures from 145 to 185° F. which is necessary to prevent the lipolysis induced by homogenization of raw milk, with extremely short heating and cooling times.

EXPERIMENTAL METHODS

The small-tube Mallory heat exchanger and the copper holding coil described previously (7) were used to process the milk. Raw whole milk was heated to 105° F. in the steam-jacketed hot well. The milk then was homogenized at a pressure of 1,500 lb. per square inch and pumped through the heat exchanger by means of the homogenizer acting also as a high pressure pump. Within 5 seconds after homogenization, the milk was heated to the desired holding temperature. After the desired holding times had elapsed, samples were drawn out of the holding coil into test tubes immersed in ice water. All samples were stored 44-48 hours at 40° F. before examination for rancid flavor and other properties.

Preliminary experiments were run to ascertain the most applicable methods for determining the extent of lipase action. Gould and Trout (4) stated that fat acidity was the most reliable criterion, but difficulty was experienced by the present authors in churning the homogenized milk and extraction methods were not considered reliable. Titratable acidity and pH measurements of the milk did not prove as sensitive as organoleptic examination. Doan and Minster (1), Halloran and Trout (5), and Tarassuk and Henderson (15) have used surface tension changes as an index of extent of rancidity. The usual surface tension of the milk used in this study was about 44 dynes/cm. at 20° C. and the surface tension of butyric acid is about 26 dynes/cm. at 20° C. Preliminary experiments indicated that surface tension changes were sufficient when rancidity developed to use this as a measure of lipase activity. These measurements always were supplemented with organoleptic examination.

Surface tension was measured with a du Nouy tensiometer at 20-21° C. Measurements were made in a constant temperature (21° C.) room after the samples had been brought to a temperature of 20° C. in a water bath. The tensiometer was standardized to absolute units with materials of known surface tension and the results are reported in dynes per centimeter. Each reported result is an average of at least three determinations whose maximum variations were within ± 0.5 dynes/cm. of the average. Organoleptic examinations were made on the same samples. The samples were scored by at least three competent judges for rancid flavor, and the results reported on the basis of 0 for no rancid flavor, ? for questionable, 1 for definite, 2 for pronounced, and 3 for very pronounced rancidity.

EXPERIMENTAL RESULTS

Raw milk was heated in 0.83 second to various temperatures within 5 seconds after homogenization, held for various lengths of time and then rapidly cooled. The changes in flavor and surface tension of the milk after 44–48 hours of storage at 40° F. are shown in table 1. Four different lots of milk were used to secure the data listed. No rancid flavor developed in any of the lots of raw unhomogenized milk in the 44 to 48-hour storage period at 40° F., and the surface tension of these lots of milk varied from 44.0 dynes/cm. to 44.7 dynes.

TABLE 1

Effect of heating milk on lipolytic action induced by homogenization of raw milk at 105° F.

Lot no.	Sample no.	Temperature	Time held	Rancid flavor ^a	Surface tension ^a
		(°F.)	(sec.)		(dynes/cm. 20° C.)
1	1a	177	0.03	2	42.0
	b	180	0.03	2	41.7
	c	184	0.03	?	42.4
	d	186	0.03	0	45.4
1	3a	160	2	2	39.9
	b	163.5	2	2	41.9
	c	167	2	0	44.0
	d	169	2	0	44.8
2	2a	164	1	3	39.5
	b	167	1	2	39.5
	c	170	1	?	43.5
	d	173	1	0	44.5
3	4a	160	2	2	40.6
	b	160	6	1	42.6
	c	160	10	0	45.4
	d	160	14	0	45.6
3	5a	155	2	3	39.3
	b	155	12	1	42.1
	c	155	22	0	44.7
	d	155	32	0	45.0
4	6a	150	33	2	40.8
	c	150	60	?	43.2
	d	150	120	0	44.0
2	7a	145	200	1	41.5
	b	145	300	?	43.2
	c	145	400	?	43.8
	d	145	500	0	44.0

^a After storage at 40° F. for 44–48 hours.

Very pronounced rancid flavor was present in each of the lots of milk which were homogenized raw at 105° F. and stored 44–48 hours at 40° F. The surface tension of the raw homogenized milk after storage ranged from 35.0 dynes/cm. to 37.1 dynes/cm.

When milk was heated sufficiently to prevent lipolytic activity, as indicated by the organoleptic examination, the surface tension of the heated milk was essentially the same as that of raw unhomogenized milk. Decreases in surface tension accompanied the rancid flavor development on those samples which were

not heated sufficiently to stop lipase activity. This is in agreement with the work of others (1, 5, 15).

From the data in table 1 and consideration of other trials in which flavor changes only were observed, the time-temperature heat treatment relationships given in figure 1 are believed to be ample for prevention of lipolytic action induced by homogenization of raw milk.

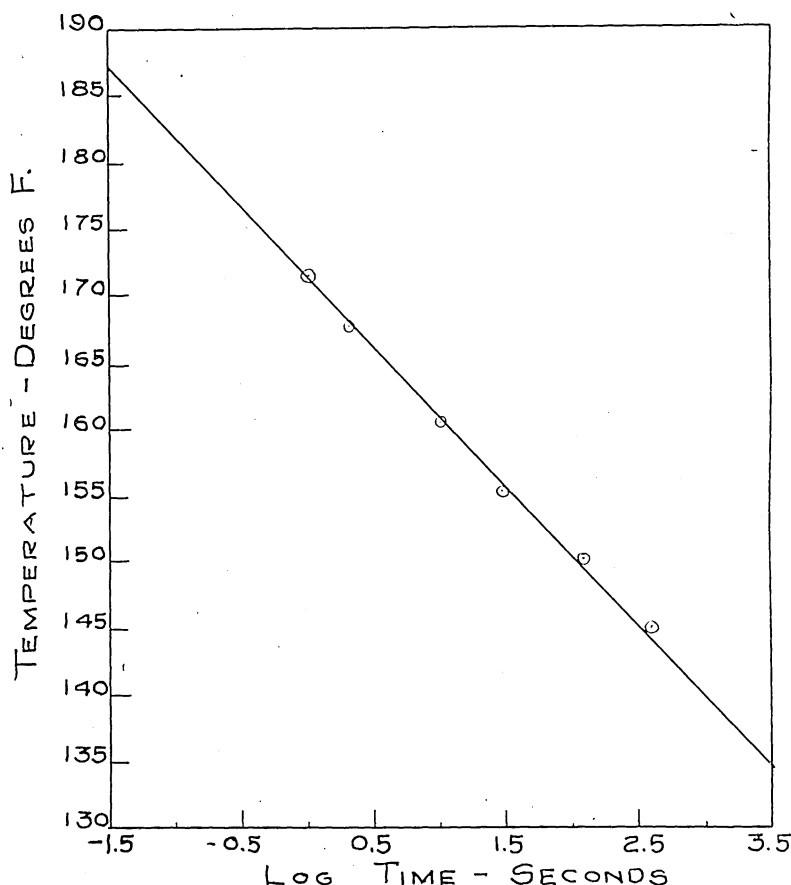


FIG. 1. Time-temperature relationship for the inactivation of the lipase enzyme in milk.

The data plotted in figure 1 show temperature to be a straight line function with log time, as was the case with the inactivation of the phosphatase (7). At 185° F. the time of inactivation is the same for both phosphatase and lipase, but the slope of the lipase curve is greater than that of the phosphatase; *i.e.*, as the temperature is lowered, lipase is inactivated with progressively less time of exposure than is the phosphatase.

If one extrapolates the curve in figure 1 to 30 minutes holding time, a temperature of 137° F. is indicated as necessary to prevent this induced type of lipolysis. Krukovsky and Sharp (10) found that a temperature of 135° F. for 30 minutes was required to prevent lipolysis induced by the warming and cool-

ing procedure of Krukovsky and Herrington (8); Tarassuk (14) found 130° F. for 30 minutes was sufficient to prevent the spontaneous type of lipolysis. Either there is a difference in the heat treatment necessary to inactivate the enzyme in the two systems, or perhaps the discrepancy could be explained by the differences in methods used to follow lipase activity or by the differences in the rates of heating to and cooling from the holding temperatures employed.

Effect of Rate of Heating

These samples were heated to the temperatures indicated within 5 seconds after homogenization. In unheated samples rancid flavor developed within 5 to 10 minutes after homogenization. With a slow rate of heating (5° F. per minute), such as would be the case if a commercial pasteurizing vat were used, the rancid flavor developed before the holding temperature was reached. If homogenization were done after heating, one could demonstrate the importance of heating rate. In one experiment, milk was heated at a constant rate of approximately 5° F. per minute and portions were homogenized as the milk was being heated. These were cooled immediately over the surface cooler and stored at 40° F. A temperature of 142° F. was sufficient to prevent the development of the rancid flavor for at least 48 hours. The results of previous experiments listed in table 1 show that a temperature of 185° F. without holding is necessary to prevent lipolysis when the heating is accomplished in 5 seconds. The effect of heat is accumulative and the temperature and holding time necessary to inactivate the enzyme will be determined by how rapidly one heats to and cools from the temperature at which the milk is being held.

Influence of Copper on Lipolysis Induced by Homogenization

Herrington and Krukovsky (6) found that additions of 0.2–0.4 p.p.m. copper reduced lipolysis almost 20 per cent. Krukovsky and Sharp (10) showed that in the absence of dissolved oxygen, copper in concentration of 2–8 p.p.m. had almost no inhibiting effect on lipolysis induced by temperature manipulation (8). Gould's (3) results showed copper to have no significant effect on the extent of lipolysis in raw milk induced by homogenization. In this study, holding of the milk at various temperatures was accomplished by use of a copper holding coil, so the milk possibly was contaminated with copper. The previous results secured in this study might not be truly representative of the effect of heat treatment alone, but might be due to the combined effects of heat and copper.

Accordingly, raw milk was heated (105° F.), homogenized, and immediately heated to 145° F. with the Mallory, as in previous experiments. The milk was collected in test tubes and immersed in a constant temperature (145° F.) bath, held for various times and cooled in ice water. Flavor and surface tension determinations were made after 44 hours of storage at 40° F. The results, as well as those secured by the copper coil holding method, are given in table 2. These results indicate that there was no appreciable difference in degree of lipolysis whether milk was held in the copper holding coil or in glass test tubes immersed in water bath. Similar results were secured at 153° F. It was found

in one trial that milk which was held in the copper coil for 10 minutes at 145° F. contained 8.5 p.p.m. copper. It is believed that this would be above the maximum amount of copper which would be present in any sample in the study because the coil had not been used for quite some time and the accumulated copper oxide was not thoroughly removed previous to the trial. The control milk not exposed to the coil contained 0.11 p.p.m. Even the 8.5 p.p.m. copper secured under the most drastic treatment is below the level (10 p.p.m.) which Gould (3) found to have no effect on lipolysis.

TABLE 2

Effect of heating homogenized raw milk to 145° F. and holding various lengths of time in test tubes vs. holding in copper holding coil on the extent of lipolysis after 44 hours of storage at 40° F.

Samples ^a	Time (sec.)	dynes/cm. (20° C.)	Rancid flavor
1	200	41.5	1.0
1a	200	43.7	1.0
2	300	43.2	?
2a	300	43.9	1.0
3	400	43.8	?
3a	400	44.4	?
4	500	44.0	0
4a	500	44.5	0
Raw	not heated	37.1	3
Raw a	" "	37.9	3

^a Sample numbers followed by *a* were held in glass tubes; others were held in copper coil.

SUMMARY

The holding times at various temperatures from 145–185° F. required to prevent lipolysis induced by homogenization of raw milk were determined. A semi-log relationship of temperature with time was observed. At 145° F., approximately one-third the time was required to inactivate the lipase as was required to inactivate the phosphatase (7), but at 185° F. the same time was required to inactivate both enzymes.

Added copper had no noticeable effect on the time-temperature relationships for inactivation of lipase under the conditions of these experiments.

The time required at any temperature to inactivate the lipase was found to vary with the rate of heating to and cooling from the holding temperature. When milk was heated at the rate of 5° F. per minute, instantaneous exposure at 142° F. was sufficient to inactivate the enzyme, while heating by means of the Mallory unit within 5 seconds required a temperature of 185° F. with instantaneous exposure.

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PROPERTIES OF THE COLOSTRUM OF THE DAIRY COW. II. EFFECT OF PREPARTAL RATIONS UPON THE NITROGENOUS CONSTITUENTS¹

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The importance of colostrum in sustaining the health of neonatal dairy calves has received renewed emphasis in recent years, yet little is known concerning factors affecting the properties of this product. One phase of a study of the relation of preparturient dairy rations to the health of the cow, to the well-being of the newborn calf, and to the properties of colostrum included an investigation of the effect of level of protein intake on concentrations of nitrogenous constituents in the mammary secretions during the postpartum transition from colostrum to milk. This phase of the investigation is reported herein.

PROCEDURES

Feeding and management of experimental animals. Twenty pregnant heifers and cows were paired according to breed and to number and stage of gestation. The heifers included two pairs of Guernseys and one pair each of Holsteins, Ayrshires and Jerseys. The cows, all in their second gestation, included two pairs each of Holsteins and Ayrshires and one pair of Jerseys. All animals calved within the 5-month period from the middle of November, 1945, to the middle of April, 1946.

Seven weeks (average) before parturition, one cow of each pair was given a high-protein ration consisting of a concentrate mixture (approximately 25 per cent crude protein), Atlas sorgo silage and alfalfa hay. The other cow of each pair was fed a low-protein ration consisting of corn, Atlas sorgo silage and prairie hay. Silage and hay were fed in the ratio of 3:1 to the extent of the appetite of each animal, and concentrates were given at the rate of 10 lb. per 1,000 lb. body weight. The same quantity of concentrate was fed throughout the trial, the initial body weights being used as a base for establishing the level of feeding. Starting on the ninth day postpartum, all cows were changed to the regular herd ration consisting of alfalfa hay, sorghum silage and a concentrate mixture containing approximately 16 per cent crude protein.

Collection of samples and analytical procedures. The calves were not allowed to nurse. The mammary secretions were withdrawn as completely as possible by standard milking methods, either hand or machine, at approximately 12-hour intervals. The first collection was made as soon as possible after parturition, usually within 4 hours. The total mammary secretions removed at each milking were well mixed before sampling. Colostrum and milk from each cow were ana-

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lyzed separately for each of the first six milkings and as composites for the seventh and eighth, the fifteenth and sixteenth, and the twenty-seventh and twenty-eighth milkings. Samples that were not analyzed immediately after collection were stored in a refrigerator at approximately 4° C. for a period not exceeding 4 days.

Rowland's method (14) was used to determine the nitrogen distribution. The only modifications of the method were the omission of selenium oxychloride as catalyst in the digestion procedure and the use of smaller samples for analysis. The amount of sample was reduced for two reasons: first, colostrum has a higher protein content than milk, which was the product for which the method was designed; and second, a micro-Kjeldahl apparatus was used in the distillation. The quantities of reagents for digestion and distillation were adjusted accordingly. Albumin and globulin nitrogen were not separated but were computed together by subtracting the values for non-protein nitrogen from those of non-casein nitrogen. Percentages of total protein and of casein were calculated from values for total nitrogen, non-protein nitrogen and non-casein nitrogen. Corrections were not applied to adjust for the volumes occupied by the precipitates of protein and fat.

RESULTS

Although considerable variation among the cows was observed, colostrum and early milk from animals of both groups had a similar total protein content and distribution of the components (fig. 1). While total protein, casein and albumin-globulin values for cows receiving the low-protein ration tended to be slightly higher during the first few milkings than were the values for corresponding samples from the high-protein group, the differences at none of the various periods were significant (*t*-test, $P = 0.05$). Concentrations of non-protein nitrogen were greater in both colostrum and early milk from cows receiving the high-protein ration than in these secretions from cows fed the low-protein ration. The differences, however, were significant only in samples representing the fourth, the fifth, the sixth, the seventh and eighth, and the fifteenth and sixteenth milkings. There was no significant difference in non-protein nitrogen of milk collected from the two groups on the fourteenth day (twenty-seventh and twenty-eighth milkings), which was 6 days after all cows had been changed to the regular herd ration.

Concentrations of the protein components tended to decrease logarithmically during the first four or five milkings of the transition period, the rate of change being similar for both groups (fig. 1). A markedly lower rate of change in the concentrations of protein components was evident by the sixth milking, the retardation seeming to occur earlier in the casein than in the albumin-globulin fraction. A subsequent gradual decline continued to the end of the second week, the time of final sample collection. The changes noted in total protein largely reflected changes in the albumin and globulin.

Non-protein nitrogen also followed a logarithmic decline which, except for the increases from the first to the second milkings, seemed to continue at approxi-

mately the same rate for the first 16 milkings. The physiological significance of the occurrence of higher values at the second than at the first milking is obscure. This increase was observed in colostrum of 14 of the 20 cows, and for each of the remaining animals the decreases from the first to the second milking were less than 0.002 per cent nitrogen.

Previous studies (6, 10), which indicate that colostrum from first-lactation cows is higher in vitamin A than that from cows in later lactations, suggested con-

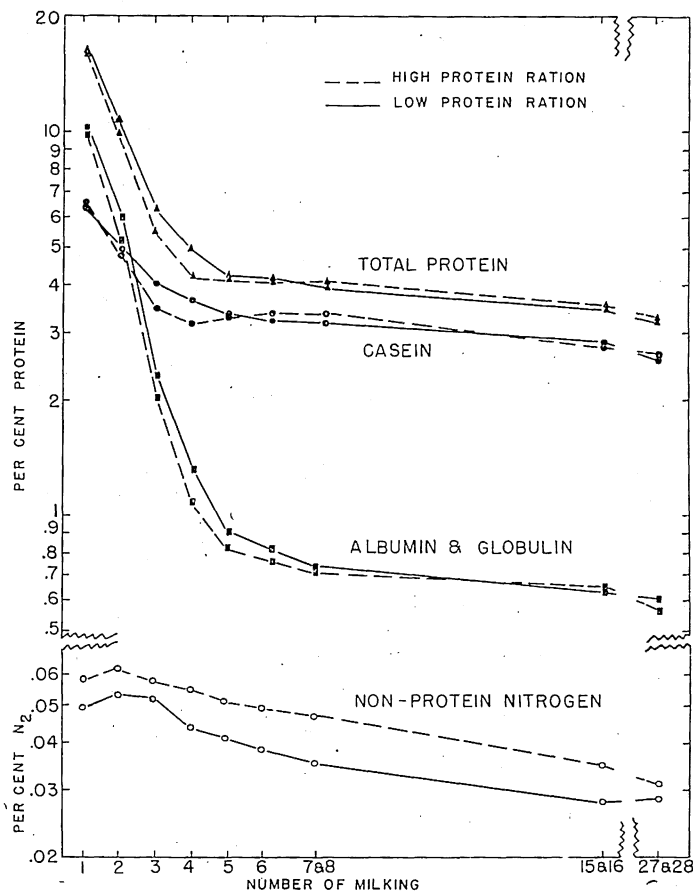


FIG. 1. Distribution of nitrogen fractions of colostrum and early milk from two groups of cows receiving high- and low-protein rations, respectively. Each group is composed of five first- and five second-lactation cows.

sideration of the relation of lactation number to the nitrogenous components of the initial mammary secretions. Analysis of the intra-group data (table 1) indicates that first-lactation cows receiving the high-protein ration secreted more albumin and globulin in the mammary products, particularly in the first three milkings, than did second-lactation cows of the same dietary group. These differences were reflected further in total protein. Concentrations of albumin-globulin nitrogen, however, were similar in the mammary secretions from first- and

TABLE 1
Distribution of nitrogen fractions of colostrum and early milk from groups of cows receiving either a high- or a low-protein ration

Ration	Lactation	No. of milking									
		1	2	3	4	5	6	7 + 8 ^a	15 + 16 ^a	27 + 28 ^a	
Total protein, %											
High protein	1 ^b	19.86 ± 2.99 ^c	13.03 ± 3.47	6.67 ± 1.39	4.52 ± 0.37	4.30 ± 0.41	4.30 ± 0.19	4.18 ± 0.29	3.60 ± 0.25	3.39 ± 0.19	
	2	12.95 ± 1.60	6.81 ± 1.41	4.30 ± 0.82	3.95 ± 0.55	3.89 ± 0.36	3.96 ± 0.35	3.85 ± 0.29	3.42 ± 0.34	3.04 ± 0.40	
Low protein	1	16.74 ± 1.73	10.95 ± 1.70	6.04 ± 1.10	4.46 ± 0.66	3.94 ± 0.32	3.90 ± 0.16	3.72 ± 0.26	3.44 ± 0.18	3.02 ± 0.38	
	2	16.29 ± 5.12	10.39 ± 4.67	6.50 ± 2.09	5.42 ± 1.19	4.50 ± 0.74	4.32 ± 0.50	4.10 ± 0.44	3.47 ± 0.59	3.30 ± 0.16	
Casein, %											
High protein	1	6.81 ± 1.64	5.44 ± 0.97	3.75 ± 0.55	3.23 ± 0.17	3.36 ± 0.26	3.47 ± 0.16	3.43 ± 0.25	2.93 ± 0.16	2.75 ± 0.18	
	2	6.24 ± 0.79	4.15 ± 1.24	3.20 ± 0.59	3.09 ± 0.42	3.17 ± 0.27	3.25 ± 0.30	3.18 ± 0.23	2.80 ± 0.26	2.56 ± 0.33	
Low protein	1	6.32 ± 1.13	5.59 ± 0.79	3.66 ± 0.72	3.23 ± 0.35	3.10 ± 0.16	3.08 ± 0.34	3.05 ± 0.20	2.82 ± 0.09	2.47 ± 0.29	
	2	6.19 ± 1.32	4.39 ± 0.72	4.31 ± 0.93	4.02 ± 0.76	3.54 ± 0.51	3.41 ± 0.44	3.30 ± 0.34	2.85 ± 0.54	2.65 ± 0.13	
Albumin plus globulin %											
High protein	1	13.06 ± 1.88	7.62 ± 2.76	2.90 ± 0.98	1.29 ± 0.31	0.94 ± 0.19	0.83 ± 0.13	0.74 ± 0.07	0.67 ± 0.10	0.62 ± 0.14	
	2	6.73 ± 1.16	2.66 ± 0.19	1.15 ± 0.24	0.86 ± 0.14	0.72 ± 0.11	0.70 ± 0.13	0.67 ± 0.08	0.62 ± 0.08	0.48 ± 0.06	
Low protein	1	10.44 ± 2.63	5.38 ± 1.33	2.31 ± 0.65	1.26 ± 0.32	0.85 ± 0.17	0.72 ± 0.13	0.67 ± 0.10	0.62 ± 0.10	0.55 ± 0.12	
	2	10.10 ± 4.86	6.52 ± 4.81	2.30 ± 1.36	1.39 ± 0.65	0.97 ± 0.27	0.90 ± 0.19	0.80 ± 0.12	0.62 ± 0.06	0.65 ± 0.13	
Non-protein nitrogen, %											
High protein	1	0.061 ± 0.007	0.066 ± 0.009	0.062 ± 0.008	0.060 ± 0.012	0.054 ± 0.006	0.052 ± 0.008	0.049 ± 0.004	0.036 ± 0.004	0.031 ± 0.003	
	2	0.055 ± 0.008	0.058 ± 0.009	0.053 ± 0.009	0.049 ± 0.006	0.048 ± 0.005	0.046 ± 0.003	0.044 ± 0.006	0.033 ± 0.006	0.031 ± 0.003	
Low protein	1	0.047 ± 0.008	0.050 ± 0.008	0.051 ± 0.009	0.046 ± 0.006	0.042 ± 0.004	0.041 ± 0.002	0.039 ± 0.005	0.029 ± 0.002	0.032 ± 0.003	
	2	0.052 ± 0.015	0.058 ± 0.015	0.050 ± 0.010	0.041 ± 0.009	0.039 ± 0.008	0.035 ± 0.007	0.031 ± 0.006	0.026 ± 0.003	0.024 ± 0.010	

^a Composite samples.

^b Five cows in each group.

^c Standard deviation.

second-lactation cows receiving the low-protein ration. Furthermore, albumin-globulin values for both heifers and cows of the latter group were between those of the two groups receiving the high-protein ration. Only small differences were noted between the casein concentrations of colostrum of first- and of second-lactation cows in either dietary group.

Levels of non-protein nitrogen in colostrum and early milk from first-lactation cows receiving the high-protein ration were higher than those from second-lactation cows. Similar comparisons of colostrum from cows of the two lactation groups receiving the low-protein ration indicated slightly higher non-protein nitrogen values for first-lactation cows only after the first two milkings.

Deviations from the mean of values of the nitrogenous constituents (table 1) are considerably greater during the early colostrum period than later, as the composition of milk approaches normal, further indicating that colostrum is a more variable product than milk.

As might be expected from studies of normal milk (9), data (not shown) suggested that Guernseys and Jerseys produced colostrum of a slightly higher protein content than did Holsteins and Ayrshires. It is recognized, however, that too few animals were used to warrant conclusions relative to breed differences.

Observations of the development and condition of the mammary glands were made in conjunction with the present study. The severity of edema, as determined by palpation and by macroscopic examination, was more pronounced in heifers than in cows and did not seem to be associated primarily with the prepartal rations the animals received (17). Attempts to correlate the total protein and the albumin-globulin contents of early colostrum with the degree of edema were successful only to the extent that the average of each of these protein fractions was higher in colostrum from the ten cows judged to have the more severe edema than from the ten cows with the less severe edema. It also was found that rate of decline of total protein and of albumin-globulin contents in mammary secretions during the transition period was not related to degree of mammary congestion.

DISCUSSION

The results presented herein are in accord with previous reports indicating that the casein and the albumin and globulin contents of colostrum decrease as it changes to normal milk (3, 4, 5, 13, 15). Grimmer (5) pointed out that the proteins of colostrum tend to decrease according to a logarithmic curve during the transition period. In the present study, the decline persisted at the initial rate for only four to six milkings, a somewhat shorter interval than observed for tocopherols (11) and for vitamin A and carotenoids (10).

The effect of high-protein intake on the concentration of non-protein nitrogen of colostrum is similar to that reported for milk (7, 12). The increased protein consumption raised the non-protein nitrogen, not only of the colostrum but also of the blood serum (2). Determinations of the kinds of non-protein nitrogen in mammary secretions and in the blood serum were not made. Other investigators (1) found that in milk from cows fed iodinated casein, urea constituted

approximately one-half of the non-protein nitrogen; whereas in blood plasma, urea frequently accounted for two-thirds or more of the non-protein nitrogen. Results from feeding high- and low-protein rations to cows in normal milk production indicated that a major portion of the increase of non-protein nitrogen of milk from cows fed the former ration was attributable to urea (12).

Differences in the albumin-globulin concentrations in colostrum from first- and second-lactation cows receiving the high-protein ration are too large to be dismissed as a chance occurrence, but interpretation of results is obscured by the fact that similar differences did not occur in colostrum from the two lactation groups receiving the low-protein ration. Although protein quality is not considered to be an important nutritional factor in the case of ruminants, the fact that the proteins in the two diets were not the same might have contributed to differences in the nitrogen fractions of colostrum from cows of the two experimental groups.

Antibodies, which are believed to be important for the well-being of the newborn, are transmitted from cow to calf through the globulins of colostrum (8, 16). Hypoproteinemia is reported to cause a decrease of antibodies and a lowered resistance to infection (16). This observation raises the question of whether first-lactation cows receiving the high-protein ration provided increased amounts of antibodies along with the high levels of albumin-globulin nitrogen of their colostrum.

SUMMARY

Twenty pregnant heifers and cows were paired according to breed and to number and stage of gestation. For 7 weeks (average) before parturition, one group received a high-protein ration and the other a low-protein ration. The rations did not effect a significant difference in the levels of total protein, of casein, and of albumin-globulin fractions of colostrum and early milk from the foregoing groups. Non-protein nitrogen levels were higher in colostrum and early milk from cows of the high-protein group, but the differences were significant only in samples after the first three collections postpartum.

The decrease in concentrations of the protein fractions of mammary secretions during the transition period tended to follow a logarithmic curve for the first four to six milkings, after which the rate of decline was less rapid. Changes in non-protein nitrogen seemed to continue at approximately the same logarithmic rate in samples representing the second through the sixteenth milkings. Rates of change of the nitrogenous constituents were similar in colostrum and in milk from cows receiving either the high- or the low-protein rations.

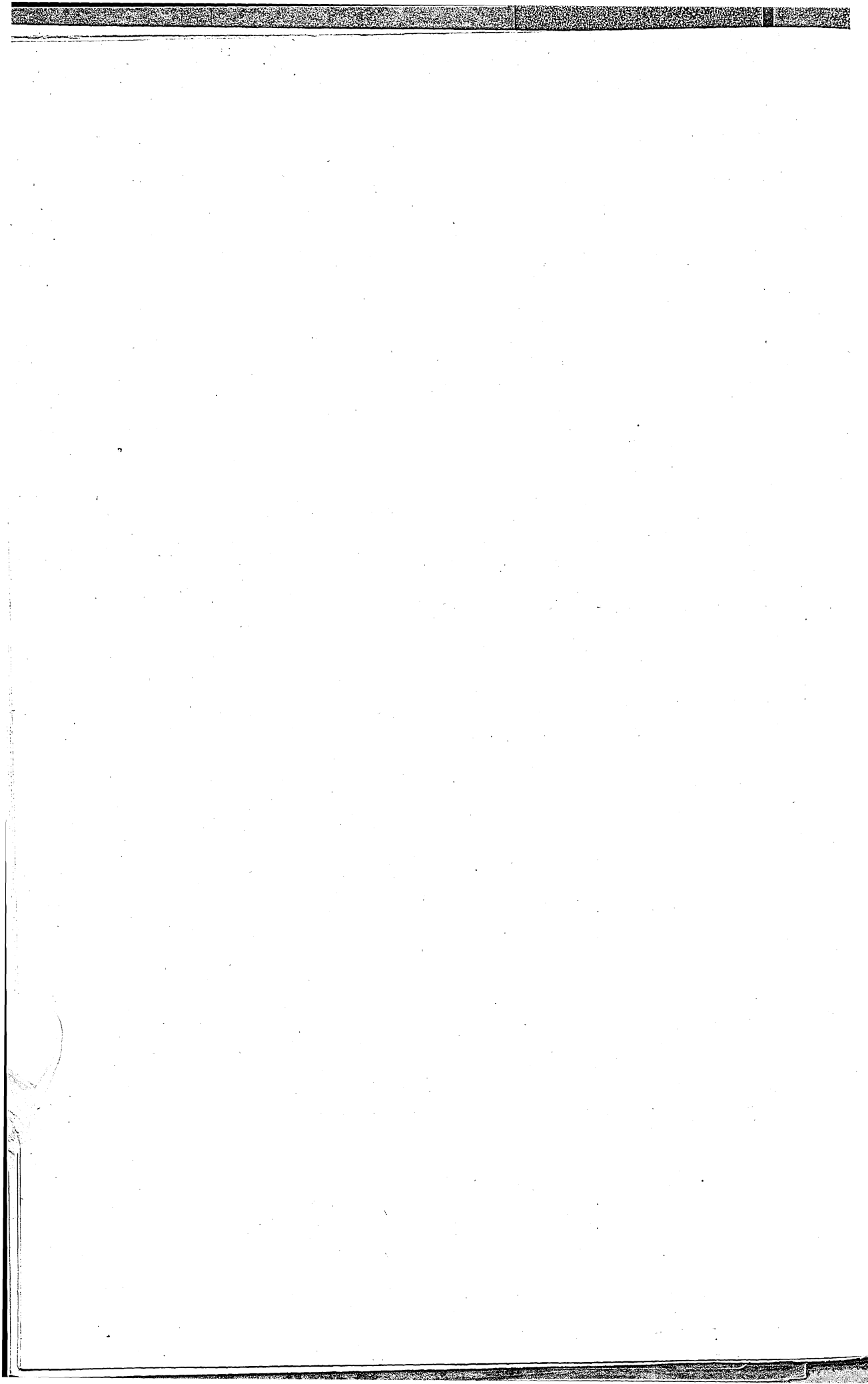
Analysis of intra-group data indicated that colostrum from first-lactation cows receiving the high-protein ration contained higher levels of albumin-globulin nitrogen than did second-lactation cows receiving the same ration. Only small differences were observed after the first four milkings. Similar differences between heifers and cows fed the low-protein ration were not evident; the values for both of these lactation groups were between those of the heifers and cows receiving the high-protein ration.

Deviations from the mean of values for nitrogen fractions for individual cows within the various groups were considerably greater during the early colostrum period than later, as the composition of milk approached normal.

Total protein and albumin-globulin contents of early colostrum were related to degree of mammary edema only to the extent that the averages of each of these protein fractions were higher from the ten cows judged to have the more severe edema than from the ten cows with the less severe edema. The rate of decline of total protein and of albumin-globulin contents of mammary secretions during the transition period was not related to the degree of mammary edema.

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THE VALUE OF WINTER PASTURE AND SWEET POTATO MEAL FOR LACTATING DAIRY COWS¹

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During recent years, considerable attention has been devoted to the value of winter grazing in the South as a means of reducing feed costs. In many of the southern states, it is estimated that the acreage utilized for winter pasture crops has more than doubled during the past 5 years. This important trend is destined to have a significant influence on the future of the dairy industry in the South.

Therefore, the present study was made in an effort to secure information about the comparative economic and physiologic value of winter grazing versus dry roughage feeding for lactating dairy cows under Georgia conditions. Furthermore, the experiment was so designed that additional information also could be secured on the feeding value of sweet potato meal. A previous study (5) showed no significant difference in the milk and butterfat production or in the liveweights of dairy cows when they were fed a grain mixture consisting of 36 per cent corn or sweet potato meal. Sweet potato meal also was observed to be as palatable as corn when fed in this proportion.

REVIEW OF LITERATURE

Various investigators have pointed out some of the beneficial effects of good grazing upon the quantity (13, 15) and quality (2, 3, 4, 6, 7, 9, 10, 11, 16, 17) of milk produced and the economic aspects (12, 18) of milk production during the seasons of the year when pasture can be provided.

Neel (13) calculated that Balboa rye provided grazing on an average of 169 days per winter in certain sections of Tennessee. A crimson clover and rye grass winter pasture at the University of Georgia Dairy Farm carried at least one cow per acre for 198 days during the winter months of 1946.

Hodgson *et al.* (8) observed an increase in milk production of about 25 per cent when the cows were changed from dry roughage feeding to pasture.

The work of Smith (18) showed that the dairy cow obtained 84.1 per cent of her total feed requirements from grazing during the pasture season of 204 days in the limestone area of southern Indiana. One hundred lb. of digestible nutrients were furnished by silage and hay at a cost of 92 cents and \$1.08, respectively. On the other hand, 100 lb. of digestible nutrients were obtained from pasture at an average cost of 20.1 cents. Calculated on the average cost per 100 lb. of digestible nutrients of feeds other than pasture fed to all livestock

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over a 5-year period, permanent pasture provided \$8.00 worth of feed at a cost of \$1.56 per acre or 19.7 cents per 100 lb. of digestible nutrients.

In studies conducted by the United States Department of Agriculture (17), pasture furnished about 33 per cent of the total nutrients required by cows producing market milk, while constituting only about 14.1 per cent of the total feed cost. Moore at the Mississippi Station (13) reported that the feed cost was \$1.21 per 100 lb. of milk produced when the cows were fed harvested roughages as compared with 69 cents (not including the cost of pasture) when the cows were provided pasture.

EXPERIMENTAL PROCEDURE

Fifteen cows of the University of Georgia Dairy Herd (three Guernseys and 12 Jerseys) were selected and balanced into three groups as evenly as practicable on the basis of age, breed, weight, current production, number of previous lactations, length of dry period and number of days fresh at the start of the experiment. These groups were divided further into five similar outcome groups. The cows were fed identical rations (ration A) during a 14-day preliminary period prior to being placed on the experimental ration (table 1).

TABLE 1
Rations used in study

Ingredients	Ration A	Ration B	Ration C	Ration D
Concentrate				
Ground corn	200	200	200
Sweet potato meal	200
Oats	200	200	200	200
Wheat bran	100	100	100	100
Soybean meal	150
Cottonseed meal	150	150	150
Steam bone meal	6	6	6	6
Salt	6	6	6	6
Roughage				
Silage, lb.	6 per cwt.
Hay, lespedeza	none	ad lib	a lib	ad lib
Winter pasture ^a	none	8	none	none
(grazing hr. daily)				

^a Seeding rate was 5 bu. oats, 2 bu. barley and 20 lb. vetch per acre. Fertilizer (10-4-2) was applied at the rate of 200 lb. per acre at time of seeding on Oct. 28, 1946.

Rations B and C were composed of the same constituents except that grazing was provided in addition when the cows were being fed ration B. Rations C and D were of the same composition except that corn was supplied as the main source of carbohydrate in ration C, while sweet potato meal was the main source of carbohydrate in ration D.

The study was conducted during the winter of 1947 for three periods of 28 days each. A 4-day change-over period preceded each experimental period for the purpose of counteracting partially any carry-over effect that the previous ration may have caused and to give the cows an opportunity to become adjusted to the change in feed. The animals were quartered either in the stanchion barn or in a dry lot during the entire time of the study except one group which was

on a 22-acre winter pasture during the day. The pasture group (fed ration *B*) was permitted to graze approximately 8 hours each day, in addition to being fed lespedeza hay and concentrates.

Collection of milk samples. Samples of milk were collected from each cow during the 14-day preliminary period and scored for flavor for the purpose of eliminating any cow that might be giving abnormally-flavored milk. Composite evening and morning milk samples were taken from each feed group twice weekly during the experiment. The samples were placed in storage at a temperature of 40° F. and scored within 15 hours. The butterfat test of each cow's milk was determined biweekly.

Feed samples. A composite sample of the concentrate mixture fed during each experimental period was analyzed for the percentages of moisture, fiber, crude protein, ash, fat and N.F.E.

RESULTS

Physiological condition of the cows. The health and general condition of the cows throughout the study were excellent, except that two cows developed mastitis soon after the study was started. Milk from these cows was not used in the flavor study. Recurrence of this condition throughout the study made it necessary to calculate the milk and butterfat production data of these cows according to the Yates method (20).

The feces of the cows that were fed rations *C* (containing 30.2 per cent corn) and *D* (containing 30.2 per cent sweet potato meal) were firm in contrast to the loose, slightly watery feces of cows that received ration *B* (containing pasture). The liveweight data collected showed no definite trend for or against either of the three rations.

Feed consumption and palatability. All the rations were eaten readily the first time that they were offered. The total roughage consumption of the cows that were fed rations *C* and *D* was 8,003 lb. and 8,154 lb., respectively. Obviously, the small difference between the roughage consumptions of the two groups would be statistically non-significant.

When the cows were allowed to graze winter pasturage an average of 8 hours daily (ration *B*), they consumed only 4,358 lb. of dry roughage or 45.6 per cent (1.82 tons) less hay than did the cows on ration *C*. With hay selling at \$35.00 per ton, the winter grazing had an average value of \$4.25 per cow for a period of 28 days as a dry roughage supplement. This does not take into consideration the increase in milk production (to be discussed later) or the decrease in grain consumption which resulted when the cows went on pasture. The greatest percentage of the dry roughage consumed by the cows on ration *B* was eaten at night when green grazing was not available. Even a greater percentage of the nutrients would have been obtained from grazing had the pasture been seeded earlier.

Chemical analysis of feeds. The concentrate part of each experimental ration was analyzed chemically. These data (table 2) show that ration *B* and *C* (containing corn) had an average of 1.43, 0.83 and 1.05 per cent more moisture,

TABLE 2
Chemical analyses of concentrate mixtures^a

Period	Ration	Moisture	Ash	Protein	Fat	Fiber	N.F.E.
		(%)	(%)	(%)	(%)	(%)	(%)
I	B and C	9.33	4.15	16.69	4.08	8.48	57.27
	D	7.88	5.10	16.25	3.24	8.79	58.74
II	B and C	8.90	4.21	19.06	4.21	9.10	54.52
	D	8.06	5.21	18.13	3.09	10.04	55.47
III	B and C	10.86	4.75	17.69	4.48	8.97	53.25
	D	9.97	4.96	16.56	3.30	9.71	55.50
Av.	B and C	9.97	4.37	17.81	4.26	8.85	55.01
	D	8.54	5.09	16.98	3.21	9.23	56.57

^a Chemical analyses of feeds were made by the department of the state chemist.

crude protein and fat, respectively, than did ration *D* (containing sweet potato meal). On the other hand, ration *D* contained 0.72, 0.38 and 1.56 per cent more ash, fiber and N.F.E., respectively, than did rations *B* and *C*.

Milk and butterfat production. The lactation curves (fig. 1) represent the mean milk yields of outcome groups 2, 4 and 5. The data from outcome groups 1 and 3 were not included, because one of the cows in each of these groups, as pointed out earlier in the report, had recurring attacks of mastitis. A decided increase in milk production occurred in every instance when the cows were changed from dry roughage feeding to pasture. Conversely, when the animals were changed from winter grazing to dry roughage feeding, there was a sharp

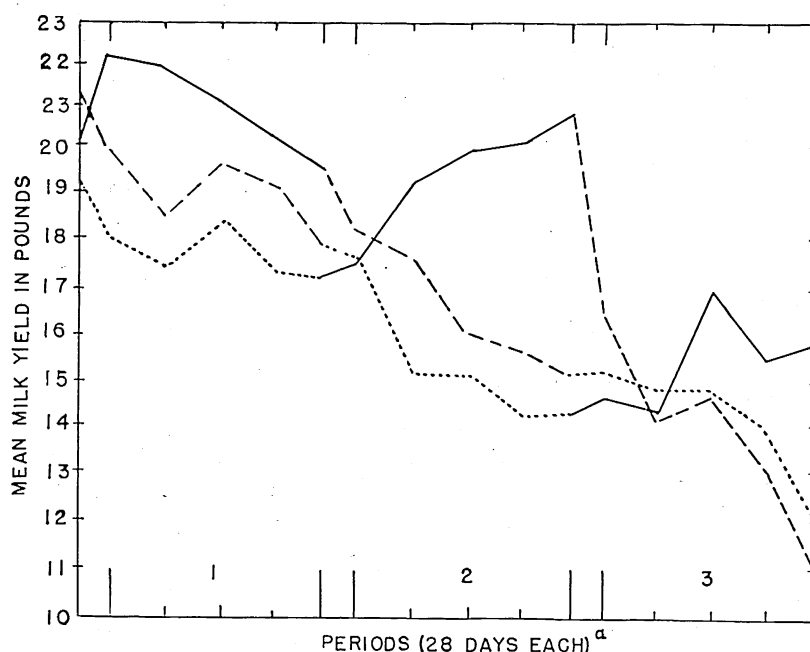


FIG. 1. Lactation curves showing the effect of the test rations on the cows during each of the experimental periods—a 4-day change-over period preceded each experimental period. (Ration B———; Ration C———; Ration D.....)

drop in milk production. The total milk production of the cows³ fed rations *C* and *D* was 8,805 and 8,618 lb., respectively, while the milk yield of those that were fed ration *B* was 10,179 lb. The milk production of the cows while on winter grazing was 15.6 per cent greater than that produced when they were fed ration *C*. The milk yield of the cows fed ration *C* was 2.2 per cent greater than that of the cows fed ration *D*.

The statistical design which made it possible to analyze the milk and butterfat production data statistically was a latin square replicated five times (1, 19). Latin squares, as used in these analyses, refer to outcome groups. Analysis of variance gave an *F*-test (37.08) which indicated a highly significant difference between rations. The mean milk yields per cow when rations *B*, *C* and *D* were fed were 679, 587 and 575 lb., respectively. Application of the *T*-table to determine the least significant deviation revealed a highly significant difference between the population mean milk yield for rations *B* and *C*. Differences in milk yields between rations *C* and *D* were non-significant. The effect of rations on butterfat production was the same as that on milk production. The butterfat yield of the cows while on winter grazing was 18 per cent greater than that produced when they were fed the same type of concentrated mixture and dry roughage. On the basis of the prevailing price (\$1.39 per lb. of grade A butterfat) at the time of the study, the increase in butterfat yield (78 lb.) in favor of ration *B* over ration *C* was worth \$102.86 for the three experimental periods or an average of \$6.86 per cow per period of 28 days. In considering the savings in dry roughage (valued at \$4.25 per cow) and the increased production (valued at \$6.86 per cow), the gross value of the pasture was \$71.75 per cow for a period of 180 days. If the cost of providing the pasture is estimated at \$35.00 per acre, the net returns to a dairy farmer as the result of including winter grazing in his feeding program would be approximately \$36.75 per cow for a period of 180 days. This is a conservative figure because a considerable decrease in consumption of concentrates occurred when the cows went on pasture.

Milk flavor scores and criticisms. The milk produced by cows on winter grazing had a very distinct feed flavor. However, the flavor was very pleasing to the taste except when the cows were milked immediately after being removed from pasture. When the cows were withheld from pasture as much as 12 hours before being milked, the milk was preferred over that produced by cows on dry rations only. No difference was noted in the effect of sweet potato meal and corn on the flavor of milk when the roughage fed was identical.

Composite milk samples were secured from each of the evening and morning milkings four times during each of the experimental periods and scored separately for flavor. The mean flavor scores of the evening composite milk samples when rations *B*, *C* and *D* were fed were 36.2, 37.6 and 36.9, respectively. Samples with no criticism were scored 40 to 45. The *F*-test for ration effect was significant at the 5 per cent level. Application of the *T*-test for least significant differences revealed a significant difference between the mean flavor score of the evening composite milk samples when the cows were fed rations *B* and *C*. The significant

³ Includes missing cow data calculated according to the Yates method (20).

difference in the flavor score of the milk from the cows fed these two rations appeared to be due to a strong grassy flavor which was characteristic of the milk drawn from cows immediately after being removed from pasture. The intensity of this flavor was not as great in the morning milk. The difference in the mean flavor score of evening composite milk samples when the cows were fed rations *C* and *D* was non-significant.

The mean flavor scores of the morning composite milk samples when rations *B*, *C* and *D* were fed were 37.4, 37.0 and 37.5, respectively. Statistical analysis revealed the differences between these data to be non-significant.

SUMMARY

Fifteen dairy cows of the University herd were used to study the value of winter pasture and sweet potato meal for lactating dairy animals during the winter of 1947. The project was conducted for three 28-day periods in accordance with the latin square design (1, 19). Analysis of variance was employed in analyzing the milk, butterfat and flavor data.

The cows that were on winter grazing consumed approximately 46 per cent less dry roughage and produced 15.6 per cent more milk and 18 per cent more butterfat than did the animals that were dry-lot fed. These differences in yields were highly significant.

The mean flavor scores of the afternoon milk samples were 36.2, 37.6 and 36.9 when the cows were fed ration *B* (containing pasture and corn), ration *C* (containing dry roughage and corn) and ration *D* (containing dry roughage and sweet potato meal), respectively. The differences in the flavor scores of the milk from cows fed rations *B* and *C* were statistically significant. The differences in the flavor scores of the milk from cows fed rations *C* and *D* were non-significant. The differences in the mean flavor scores of the morning milk samples from cows fed each of the rations were non-significant.

There was no significant difference in the amount of milk and butterfat produced or in the flavor score of the milk when the cows were fed a concentrate mixture consisting of 30.2 per cent of either corn or sweet potato meal. The animals ate one ration just as readily as the other. The sweet potato meal did not cause an excessive or objectionable laxative effect upon the digestive system of the cows.

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THE DETERMINATION OF BUTTERFAT IN ICE CREAM EMPLOYING MIXED PERCHLORIC AND ACETIC ACIDS

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The Babcock determination of butterfat in milk, cream and certain milk products such as skim milk (2) has been an established procedure for over 50 years. Probably no method of analysis has ever had a record remotely approaching the frequency with which the Babcock test has been applied in the dairy industry.

The unmodified Babcock butterfat test cannot be applied successfully to dairy products containing added sugar due to the charring action of the sulfuric acid. It was the purpose of the present work to show that the use of perchloric and acetic acids in place of sulfuric acid modifies the standard Babcock test to make it applicable to ice cream mix for the determination of butterfat. It can be applied without alteration of existing equipment and with marked improvements in speed, accuracy and simplicity. It diminishes the number of required manipulations per determination, as it is not necessary to add water and the bottle is centrifuged for only a 2-minute period. The increased cost of the mixed perchloric-acetic acid which it employs is more than justified by the saving in time and the abbreviation in operative details. Moderate variation in the amount of acid mixture used does not affect the accuracy of the test.

A mixture of 72 per cent perchloric acid and glacial acetic acid react to form two possible compounds (8), one with the ratio one molecule of perchloric acid to two molecules of acetic acid and the other compound with the molecular ratio of 1 to 1. Such mixtures are not hazardous to mix and may be stored without deterioration. At the boiling point, the acetic acid is evolved and may be thus separated from the perchloric acid. By the process to be described, no precautions other than those applied to the unmodified Babcock test are required. The usual care in the handling of strong mineral acids apply to both procedures.

Sugar is soluble in 72 per cent perchloric acid without charring. Butterfat is as insoluble in aqueous perchloric acid as it is in aqueous sulfuric acid. The proteins of milk and cream are soluble in perchloric acid. Since butterfat in the presence of 72 per cent perchloric acid tends to darken at temperatures near 100° C., thus making reading of the test difficult, it was found desirable to use a mixture of equal parts of 72 per cent perchloric acid and glacial acetic acid as a substitute for concentrated sulfuric acid in the application of the Babcock procedure to the testing of ice cream. The presence of sugar, ice cream stabilizers, flavors and egg products or chocolate does not interfere with the test.

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It is beyond the scope of the present work to give in any detail reference to former procedures which have been developed as substitutes for the original Babcock test as applied to the testing of ice cream. The literature on the subject is very voluminous (1, 2, 4, 5, 6, 7). In no published procedure was found any record of the use of perchloric acid for the purpose of modifying the Babcock test as applied to fat determination in ice cream or ice cream mix.

PROCEDURE

Apparatus and reagents. The perchloric acid-acetic acid mixture, which is the only reagent employed in this modification of the Babcock test, consists of equal parts of volume of 72 per cent perchloric acid ($\text{HClO}_4 \cdot 2\text{H}_2\text{O}$) and 95 per cent glacial acetic acid. Little heat is evolved from the mixing of these chemicals.

Standard Babcock equipment was used to measure the butterfat content by this method. Babcock 20 per cent ice cream test bottles graduated in 0.2 per cent were used throughout this study.

The Mojonnier test, a commercial adaptation of the official Roesse-Gottlieb method (3), was employed to carry out control determinations described in this work. All samples tested were evaluated both by the Mojonnier method and the perchloric-acetic acid process simultaneously and the results compared.

The perchloric acid-acetic acid process. The procedure of the test is as follows:

(a) Weigh a 9-g. sample of ice cream mix (or melted ice cream) into a 20 per cent Babcock ice cream test bottle.

(b) Add approximately 30 ml. of the acid reagent (equal parts by volume of 72 per cent perchloric acid and glacial acetic acid) to the test bottle, rinsing the adherent mix off the graduated stem of the test bottle into the body of the bottle as the acid is added. The ingredients should all be at room temperature during mixing.

(c) Digest the ice cream and acid mixture by immersion in boiling water for 5 minutes. No color forms at first, but upon heating in boiling water the mixture turns progressively tan, brown and finally a deep chocolate color. The curd is completely dissolved in 1 to 2 minutes. The mixture should be agitated two or three times during the digestion period. After 5 minutes, the fat will be found as an immiscible supernatant layer.

(d) Add enough of the acid mixture to bring the fat into the calibrated stem of the bottle.

(e) Place the test bottles in balanced pairs in a standard Babcock test centrifuge and revolve at proper speed for 2 minutes. If the centrifuge is heated to 60° C., the per cent of fat can be read as soon as the sample is removed from the centrifuge. If an unheated centrifuge is used, the test bottles should be tempered by immersion in a water bath (130°–140° F.) to the top of the fat column for 5 minutes before reading. The reading of the fat column is made in the customary manner after the addition of glymol.

(f) Contents of the test bottles should be poured into a reservoir of water and then emptied in the sink drain for disposal. The test bottle is rinsed with hot water and is ready for a second test. No coating of insoluble calcium salts ever accumulates on the inner walls of the test bottle. All mineral salts present in cream are soluble in the acid mixture used.

RESULTS

Experimental results on plain vanilla ice cream as compared with the Mojonnier test. Thirty-one different samples of plain vanilla ice cream were subjected to test. These samples were from a wide variety of commercial sources or were

experimental ice creams prepared in the University of Illinois Dairy Technology laboratory. No attempt was made to record their composition. The results are shown in table 1. The maximum deviation between the new method and the

TABLE 1

The analysis of plain ice cream and ice cream mix by the perchloric acid-acetic modified Babcock test and comparison with Mojonnier values

Sample no.	Perchloric acid method		Mojonnier method		Maximum variation from Mojonnier	Average variation from Mojonnier
	No. of analyses	Av. B.F.	Av. B.F.			
		(%)	(%)	(%)	(%)	(%)
1	10	11.33	11.22	+0.23	+0.11	
2	17	12.37	12.22	+0.23	+0.15	
3	18	9.05	9.03	+0.12	+0.02	
4	15	15.15	15.05	+0.13	+0.10	
5	7	12.03	12.00	+0.17	+0.03	
6	8	13.41	13.46	-0.26	-0.05	
7	15	11.80	11.89	-0.19	-0.09	
8	4	12.60	12.61	-0.11	-0.01	
9	4	12.15	12.22	-0.12	-0.07	
10	6	12.78	12.82	-0.22	-0.04	
11	4	11.78	11.69	+0.11	+0.09	
12	2	13.60	13.61	-0.01	-0.01	
13	5	10.56	10.51	+0.09	+0.05	
14	5	12.20	12.08	+0.22	+0.12	
15	6	10.13	10.17	+0.13	-0.04	
16	8	11.20	11.08	+0.22	+0.12	
17	4	10.68	10.65	+0.15	+0.03	
18	4	12.18	11.89	+0.31	+0.29	
19	37	12.42	12.49	-0.19	-0.07	
20	8	12.42	12.30	+0.26	+0.12	
21	8	12.48	12.44	+0.16	+0.04	
22	6	12.37	12.29	+0.11	+0.08	
23	12	12.51	12.21	+0.49	+0.30	
24	10	12.62	12.28	+0.37	+0.34	
25	10	12.70	12.43	+0.37	+0.27	
26	10	12.77	12.54	+0.36	+0.23	
27	8	12.91	12.64	+0.36	+0.27	
28	4	12.33	12.31	-0.11	+0.02	
29	4	11.98	12.02	-0.12	-0.04	
30	4	11.63	11.60	+0.10	+0.03	
31	4	11.13	11.20	-0.20	-0.07	
Summary	267					+0.07

Mojonnier process was +0.49 per cent. The average algebraic difference was +0.07 per cent.

Eight analyses of the same sample gave 11.2 per cent for six determinations, 11.1 for one determination and 11.3 for the remaining test. The Mojonnier test for this sample was 11.08 per cent.

The determination of butterfat in chocolate ice cream. The procedure as described was applied to the determination of butterfat in eight samples of chocolate ice cream with the results given in table 2. Control analyses were carried out using the Mojonnier method. Results of the test of chocolate ice cream samples indicate that the perchloric acid-acetic acid procedure is satis-

factory for use in the determination of butterfat in chocolate ice cream. The average variation between the two methods was -0.11.

SUMMARY

A new reagent has been described for use in a modified Babcock butterfat analysis of plain ice cream and chocolate ice cream. The reagent consists of a mixture of equal parts by volume of 72 per cent perchloric acid and glacial acetic acid. The test requires only one centrifugation and a complete analysis can be accomplished in about 8 minutes. The results are in close agreement with those obtained by the Mojonnier method.

TABLE 2
*The determination of butterfat in chocolate ice cream
by the perchloric-acetic acid procedure*

Sample no.	Perchloric acid method		Mojonnier method		Maximum variation from Mojonnier	Average variation from Mojonnier
	No. of analyses	Av. B.F.	Av. B.F.			
		(%)	(%)	(%)	(%)	(%)
1	7	14.36	14.37	-0.17	-0.01	
2	12	13.28	13.44	-0.54	-0.16	
3	2	20.05	20.14	-0.14	-0.09	
4	5	11.00	11.07	-0.17	-0.07	
5	4	10.10	10.01	+0.19	+0.09	
6	5	15.42	15.12	+0.48	+0.30	
7	7	10.89	11.10	-0.30	-0.21	
8	7	12.53	13.26	-0.86	-0.73	
Summary	49					-0.11

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THE NUTRITION OF THE NEWBORN DAIRY CALF. II. EFFECT OF DIETARY TRYPTOPHAN ON THE URINARY EXCRETION OF NIACIN AND ITS METABOLITES BY YOUNG DAIRY CALVES

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Following the reports (6, 7) that tryptophan can replace niacin in affecting growth of laboratory animals on niacin-deficient rations, various investigators determined the effects of dietary tryptophan on the excretion of niacin and its metabolic products not only in the rat (10, 13) but also in the pig (8), horse (12) and man (11). In all cases the feeding of tryptophan resulted in a marked increase in the excretion of these substances, indicating a metabolic relationship between tryptophan and niacin. The nature and concentration of the excreted products suggest that there is a species variation in this respect. It has been found that there are large increases in the excretion of N¹-methylnicotinamide when tryptophan is fed to rats (13), pigs (8) and humans (11), whereas in the case of the horse (12), there is no significant increase in the excretion of this substance but an increase in free nicotinic acid and other non-methylated products.

In a previous communication (14) from this laboratory, a two-fold increase in the blood tryptophan of calves during the first 3 days of post-natal life was reported. This increase resulted from the ingestion of colostrum which was found to contain an average of 3.85 mg. of tryptophan per g. at the first milking. This is approximately five times that of normal milk on a wet-weight basis. In view of the existing knowledge that a metabolic relationship exists between tryptophan and niacin in the nutrition of the rat, pig, horse and man, and that calves do not require a dietary source of niacin when fed a synthetic milk diet (5), it was considered desirable to study the effects of feeding tryptophan to calves on a milk diet on the excretion of niacin and its derivatives.

EXPERIMENTAL PROCEDURE

Two calves were selected for this experiment and maintained on an exclusive milk diet from birth throughout the experimental period. Calf A, a Holstein, was put on the experiment 24 hours after birth. Calf B, a Guernsey, was assigned to the experiment at 40 days of age. This calf had been kept from the time of birth in a wire-bottomed pen. The usual procedure of feeding colostrum for the first few days was followed in both cases. When the urinary excretion of niacin and its metabolites was found to be fairly constant, each calf was fed 15 g. of L-tryptophan during a 3-day period. The weighed amount of L-tryptophan (2.5 g.) was dissolved in a small amount of dilute sodium carbonate

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solution and carefully mixed with the milk before each feeding. The calves were fed twice daily at 12-hour intervals.

Twenty-four-hour samples of urine were collected after 7.5 g. of L-tryptophan had been fed and again immediately following the last feeding when a total of 15 g. had been fed. Similar urine collections were made 2 and 7 days following the cessation of L-tryptophan feeding.

The amount of tryptophan in the milk consumed was determined each day and the amounts of niacin, other non-methylated products, N¹-methylnicotinamide and tryptophan were determined in each urine sample collected. N¹-methylnicotinamide was determined fluorometrically by the method of Huff *et al.* (4). A Coleman photofluorometer was used for the fluorescence measurement. Niacin and its non-methylated products were determined by the chemical procedure of Perlzweig *et al.* (9) with minor modifications. The *p*-dimethylaminobenzaldehyde method of Bates (1) as modified by Graham *et al.* (2) was used for the determination of tryptophan in both milk and urine. The Evelyn Photoelectric Colorimeter with appropriate filters was used for all determinations except N¹-methylnicotinamide.

RESULTS AND DISCUSSION

The effects of feeding L-tryptophan on the excretion of niacin and its metabolic derivatives are shown in table 1. It will be noted that following the ingestion of colostrum the excretion of N¹-methylnicotinamide was fairly high (table 1, calf A) but dropped rapidly, whereas the non-methylated products

TABLE 1
The effects of feeding L-tryptophan on the urinary excretion of nicotinic acid and its metabolites in dairy calves

Age	Tryptophan in the milk consumed	Urinary excretion			
		Nicotinic acid	Other non- methylated metabolites	N ¹ -methyl- nicotinamide	Total excretion
(days)	(g./day)	(mg./day)	(mg./day)	(mg./day)	(mg./day)
Calf A, born April 7, 1948					
2	9.86	1.30	2.10	5.18	8.58
5	3.19	1.22	4.06	4.85	10.53
12	3.45	3.51	13.51	1.67	18.69
14 ^a	3.96	3.38	14.17	1.91	19.46
16	4.50	3.84	32.79	2.65	39.28
18	3.62	3.62	48.04	3.35	55.01
21	4.05	4.05	34.01	2.55	40.61
27	4.30	3.10	12.10	2.54	17.74
Calf B, born February 25, 1948					
41	2.03	0.43	5.82	3.12	9.36
48	2.61	0.80	4.30	1.22	6.32
58 ^b	2.16	0.30	1.96	1.54	3.80
60 ^a	1.86	0.42	2.13	1.52	4.07
62	2.58	2.50	13.70	1.92	18.12
63	3.41	3.15	22.95	1.35	27.45
66	3.05	1.27	5.83	1.39	8.49
69	3.20	0.84	3.61	1.70	6.15

^a Following this collection 5 g. of L-tryptophan was fed daily for the next 3 days.

^b Scoured.

increased appreciably. The difference between calves in the level of free non-methylated products excreted prior to the tryptophan feeding is not explainable, although it must be remembered that these calves differed in breed and age and in milk consumption. Therefore calf *A* received a larger daily amount of tryptophan.

Following the feeding of 15 g. of L-tryptophan, there was a three- to four-fold increase in the excretion of total nicotinic acid. There was no significant increase in the excretion of free niacin or N¹-methylnicotinamide. The major portion of the increase was in the non-methylated products, and the maximum increase was noted in the collection immediately following the L-tryptophan feeding period. The data indicate that N¹-methylnicotinamide is not the main metabolic product excreted by calves. In this respect, the calf is similar to the horse (3) but different from the rat, pig and man. The Illinois workers (5) found a relatively constant excretion of N¹-methylnicotinamide in calves regardless of whether or not niacin was added to their diet. Following the cessation of L-tryptophan feeding the excretion of total nicotinic acid returned to normal for the individual in 3 to 7 days.

The results of the urinary excretion of tryptophan are presented in table 2.

TABLE 2
The effects of feeding L-tryptophan on the urinary excretion of tryptophan

Calf <i>A</i>			Calf <i>B</i>		
Age	Tryptophan in the milk consumed	Tryptophan excreted in the urine	Age	Tryptophan in the milk consumed	Tryptophan excreted in the urine
(days)	(g./day)	(mg./day)	(days)	(g./day)	(mg./day)
12	3.45	24.2	58	2.16	67.1
14 ^a	3.90	25.5	60 ^a	1.86	72.8
16	4.5	53.8	62	2.50	118.1
18	3.62	65.2	63	3.40	114.5
21	4.01	52.8	66	3.05	103.2
27	4.30	32.2	69	3.20	82.5

^a Following this collection 5 g. of L-tryptophan was fed daily for the next 3 days.

Feeding 5 g. of tryptophan daily for 3 days resulted in an increase in the excretion of tryptophan in urine. This increase could account for only 1 to 1.5 per cent of the intake. It reasonably can be presumed that most of the ingested tryptophan was utilized in the body.

The facts that both colostrum and milk are poor in niacin but relatively rich in tryptophan, and that there is a marked increase in the urinary excretion of niacin and its derivatives following the ingestion of tryptophan suggest that dietary tryptophan serves as a precursor of the niacin required by the calf.

SUMMARY

Five g. of tryptophan were fed daily for 3 days to each of two dairy calves that had been maintained from birth on a whole milk diet. The amount of urinary excretion of nicotinic acid and its derivatives and tryptophan was determined on 24-hour samples and compared with similar data obtained previous to and following the tryptophan feeding.

Tryptophan feeding resulted in a three- to four-fold increase in the excretion of total free and combined nicotinic acid. There was little change in the excretion of free nicotinic acid and N¹-methylnicotinamide. The major portion of the increase was in the non-methylated products. The data indicate that N¹-methylnicotinamide was not the main metabolic product excreted by calves.

The urinary excretion of tryptophan accounted for only 1 to 1.5 per cent of the intake.

Increase in dietary tryptophan results in increased urinary excretion of total nicotinic acid, indicating that tryptophan serves as a precursor of niacin in the young calf as in other mammals thus far studied.

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THE OXIDIZED FLAVOR IN MILK AND DAIRY PRODUCTS: A REVIEW

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The oxidized flavor discussed in this review has been known by a number of descriptive terms. The terms most commonly used to describe the flavor are "cappy," "cardboard," "emery," "metallic," "oily," "oxidized" and "tallowy." The so-called tallowy flavor which develops in fluid milk is not the same as the tallowy flavor which develops in dried milk. The former generally is believed to be caused by an oxidation of the phosphatides, while the latter is caused

by an oxidation of the glycerides. The terms "cappy" and "cardboard" were used because early workers thought that the milk bottle cap was the source of the flavor, while other terms were used in an attempt to describe the taste. The presence of this flavor in milk has caused considerable concern in Europe for a number of years. More recently, it has become of increasing importance in the United States.

The development of the oxidized flavor in milk seems to be the result of a mild chemical oxidation of a minor constituent associated with the fat. A number of authors have presented data which indicate that an oxidation of the phospholipids is responsible for the development of the oxidized flavor. Hereafter, the oxidized flavor will be referred to as *the flavor*.

Golding and Feilman (73) were probably the first to make a study of the flavor in milk. However, Guthrie (88) refers to the work of White who, in 1901, scored butter which had a metallic flavor. The defect observed by White may have been due to active glyceride oxidation, because a metallic flavor usually precedes a tallowy flavor in butter. In this early publication, the author made a thorough study of the development and inhibition of the flavor in milk, skim milk, cream and buttermilk.

THEORIES

The enzyme theory. This theory was proposed by Kende (121) and accepted in whole or in part by the workers in this field for a number of years. He claimed to have isolated an enzyme which he called "oleinase", because it catalyzed the oxidation of the oleic radical of the fat. Sharp *et al.* (192) and Chilson (37) observed that heating milk destroyed an enzyme which oxidized ascorbic acid and at the same time destroyed the enzyme which promoted the development of the flavor. The fact that the flavor increases with decreased storage temperature and the observation that the addition of ascorbic acid (a normal constituent of milk) inhibits development have been used as arguments against this theory. The observation that the flavor may be promoted or inhibited by small changes in pH and E_h also seems to be an argument against the enzyme theory.

The chemical oxidation theory. The conditions under which the flavor develops are those that promote oxidation, such as the presence of air and contamination by metals that are known to be oxidation catalysts. The optimum conditions for development of the flavor are those that cause a mild oxidation, such as low storage temperatures, a limited supply of oxygen, a low concentration of certain oxidizing agents and a limited increase in E_h . It has been observed by many workers that if the intensity of oxidation is too great the flavor will not develop (83, 130, 131). Greenbank (83) postulated and presented data in support of his assumption that the flavor is the result of an intermediate oxidation product, and that the development of the flavor in milk may be inhibited by reducing or oxidizing agents. Recently, Krukovsky and Guthrie (131) have presented data which can be used to confirm the work presented by Greenbank (83). By the careful addition of hydrogen peroxide, these authors were able to promote or inhibit the development of the flavor. Milk so treated to inhibit the

flavor development then was made susceptible by the addition of ascorbic acid. This was repeated a number of times with the same result, but eventually the addition of ascorbic acid had no effect.

The addition of copper, a mild oxidizing agent, promotes development of the flavor, while a low concentration of ferric iron may promote the development of the flavor and a higher concentration inhibit it (83). The addition of reducing agents may inhibit development of the flavor or remove the flavor once it is formed (83). In the latter case, the flavored compound must be reduced before it has become bound to the fat or protein.

THE EFFECT OF THE CHEMICAL AND PHYSICAL PROPERTIES OF THE MILK

The chemical properties of the milk which have been considered as affecting the development of the flavor are E_h , pH, poisoning action and titratable acidity.

Oxidation-reduction potential (E_h). Tracy *et al.* (212) were among the first workers to show a relationship between the development of the flavor and the E_h . They found that the addition of copper caused an increase in E_h of the susceptible samples. These conclusions were verified by Thurston (204), Greenbank (82, 83), and Webb and Hileman (223). The latter were unable to find any relationship between the E_h and the development of the flavor when copper was added to milk from individual samples. The inhibition of the flavor by bacterial growth (15, 202, 203) and by heat has been attributed by Greenbank (83) to the lowering of the E_h . Greenbank's work has been confirmed by Josephson and Doan (119), and Gould and Sommer (78). Larsen *et al.* (133) found no correlation between the E_h and the inhibiting effect of homogenization. The addition of copper and iron to milk may cause a change in the E_h . Copper and ferrous iron are most effective in promoting the flavor (83). Ferric iron and hydrogen peroxide may inhibit if a sufficient concentration is employed (83). The use of poor feed increases the E_h and promotes development of the flavor, while green feed lowers the E_h and development of the flavor is inhibited.

Poising action. Poising is the resistance of milk to a change in E_h ; it is analogous to buffering in the acid-base system. Greenbank (83) concluded that the variation in individual samples is a result of differences in poisoning. When poisoning is used as a criterion, according to Thurston's (205) classification, spontaneous milks are those which are very poorly poised, susceptible milks those poorly poised, and non-susceptible milks those well poised. The difference in poisoning between susceptible and non-susceptible samples seems to be confirmed by the data of Krukovsky and Guthrie (130) on the oxidation of ascorbic acid in susceptible and non-susceptible samples. Greenbank (81) proposed a test to detect susceptibility based on poisoning, which he claimed was 90 per cent accurate. Webb and Hileman (223) were able to predict with a fair degree of accuracy the susceptibility of samples by the rise in E_h after the addition of copper. The reduction of methylene blue in milk by light has been used as an indication of susceptibility (1, 62). Greenbank and Holm (86) have shown that methylene blue dissolved in butterfat is reduced by light.

Hydrogen ion concentration (pH). The effect of pH on the development of the oxidized flavor has not been studied extensively. Greenbank (83) found that an increase in pH of 0.1 was sufficient to inhibit development of the flavor for 24 hours. Although all samples developed the flavor after storage for 24 hours, the samples with increased pH developed less flavor. As a rule, an increase in OH ions accelerates oxidation and may prevent the development of the flavor (83). Anderson (4) presented similar data but attributed inhibition to the activation of an enzyme which destroys the flavor rather than to catalysis of oxidation by OH ions.

Titrateable acidity. Brown and Dustman (22), in a study of 220 samples of milk, were unable to find any correlation between the titrateable acidity and the development of the flavor when the milk was contaminated with copper. Anderson (4) found a relationship between the titrateable acidity and the development of the flavor. Anderson and Triebold (2) observed that reducing milk of high acidity to 0.145 per cent acidity or lower was effective in inhibiting the flavor. Winter milk generally has a higher titrateable acidity than summer milk, which would appear to be a positive correlation with the observation that winter milk is more susceptible than summer milk, but there probably are other changes that are more significant (4).

Color. The variation in the color of milk, especially the yellow color, has been correlated with the development of the flavor. The attempt to correlate color and the development of the flavor is probably a result of the fact that carotene has been thought to be an antioxidant. Anderson (6, 7) and Anderson *et al.* (10) were among the first to find a relationship between color and flavor. The yellow color of milk largely is due to the pigment carotene. Tucker *et al.* (220) found a good correlation between the intense yellow color and good flavor. Whitnah *et al.* (228) found that milk which was below the average color for the breed developed the flavor. However, they also found samples low in color which did not develop the flavor. See "Carotene," and "green feed" for additional discussion.

THE EFFECT OF MILK CONSTITUENTS

Glycerides. The glycerides of the fatty acids are reasonably stable at temperatures most conducive to the development of the flavor. When the glycerides oxidize, there is a measurable decrease in the iodine value. Kende (121) and Dahle (43) found a decrease proportional to the intensity of the flavor. Brown *et al.* (23) were unable to find any decrease. This has been confirmed by Swanson and Sommer (201). Since the flavor is thought to be the result of an oxidation of the phospholipids, no appreciable decrease in the iodine value should be expected, since the concentration of phospholipids in milk fat is low. The same authors found that the oxidized flavor is pronounced in butter, buttermilk, cream and milk, but found only a trace in butteroil. They found that oxidized butteroil when dispersed in skim milk has an off flavor but not an oxidized flavor. Dahle (44) mixed cream or butterfat with skim milk from cows that gave susceptible

milk and the flavor developed, but it developed more rapidly in the sample prepared with cream than in the one prepared with butteroil.

Phospholipids. Whole milk contains from 0.0038 to 0.2889 per cent phospholipids, according to Panzer (159). Early workers pointed out that the flavor developed most rapidly in the cream layer. Guthrie (88), as early as 1916, found that buttermilk from a susceptible cream developed a much stronger flavor than either the cream or the milk. The phospholipid content of milk and milk products (176) decreases in the following order: buttermilk > cream > whole milk > skim milk,—which has also been found to be the order of decreasing intensity of flavor development (88, 207). Thurston *et al.* (208) conclude that the oxidation of lecithin is the cause of the flavor. They arrived at this conclusion because removal of the hulls from milkfat globules, which then were redispersed, resulted in a milk which did not develop the flavor. Roland and Trebler (179) found a decreased sensitivity to copper-induced flavor when mechanical separation was employed. They attributed this to a redistribution of the lecithin between the fat and aqueous phases. Gould *et al.* (77) found no relationship between the lecithin content of the milk and the development of the flavor. Evans (60) found that lecithin (probably impure) was an antioxidant, an observation which has been confirmed by Holmes *et al.* (107) and Koenig (125). Ritter and Nusbaumer (174) found that both lecithin and cephalin of plant origin act as antioxidants. Olcott and Mattill (155) report that lecithin is not an antioxidant, but cephalin acts as such. Much of this work was done on fat substrates and may not be of great value here. Dahle and Palmer (53) conclude that spontaneous flavor, *i.e.* without metallic contamination, is due to the oxidation of the phospholipid fraction of the fat globule membrane. Josephson and Doan (119) found that a typical oxidized flavor develops when phospholipids and copper in suspension are heated together. Phospholipids plus protein developed the flavor without heat, but the intensity was greater when heat and copper were used. Swanson and Somner (201) found a decrease of 30 per cent in the iodine value of the phospholipids from milk which had developed the flavor. The data presented in these studies seem to indicate that oxidation of the phospholipids is the cause of the flavor.

Carotene. Milkfat contains 0.20 to 0.86 mg. of this yellow pigment per 100 g. of fat (176). The reason for the study of the effect of carotene on the development of this flavor is probably its purported antioxygenic activity. Briggs (20), Koenig (126) and Newton (154) conclude that carotene is an antioxidant, while other workers (19, 21, 87, 101, 156) conclude that it has no effect or is a prooxidant. However, most of this work has been done on glyceride substrates and may not be applicable in this work.

Brown *et al.* (30) concluded that some substance associated with the carotene is responsible for the development of the flavor. Trout and Schied (219) found no relationship between the carotenoid content and development of the flavor.

Vitamin A. The concentration of vitamin A in milk varies from 2.5 to 50.0 Sherman units per g. depending on the feed. It is practically all in the butterfat.

Booth *et al.* (18) found the concentration of vitamin A in summer milk was three times as great as in winter milk. Garrett *et al.* (67) found that feeds which increase vitamin A also may increase ascorbic acid. For additional discussion see "Green feed," and "supplements."

Ascorbic acid. In this review, the interest is greater in ascorbic acid than in vitamin C because the latter contains dehydroascorbic acid which does not influence the flavor (131). Milk has been found to contain as high as 26.5 mg./l. of ascorbic acid, according to Riddell *et al.* (168). It is reasonable to assume that ascorbic acid plays some role in the development of the flavor, because it is a reducing agent and has been reported to be an antioxidant. The ratio of the reduced form to the oxidized form may reflect the E_h of the milk because the ascorbic-dehydroascorbic acid system is reversible. Garrett *et al.* (67) found a relationship between the ascorbic acid content and the flavor of milk the day it was drawn. Hand and Sharp (98) found a good correlation between the oxidation of ascorbic acid and development of the flavor. Trout and Gjessing (217) found the ascorbic acid content greater in summer than in winter, which is the opposite of seasonal variation of the flavor intensity. This might indicate that ascorbic acid inhibits development of the flavor.

Whitnah *et al.* (228) found that the relationship between ascorbic acid content and development of the flavor varied in milk from different breeds. This is discussed under "Biological Factors." They found no relationship between vitamin C and development of the flavor in milk from cows within the breed. Sharp *et al.* (192) and Dahle (42) report there is such a relationship. Tucker *et al.* (220) found that a concentration of from 15 to 18 mg./l. was required to impart a good flavor to milk. Brown *et al.* (29) found that feeding KI reduced the ascorbic acid but had no effect on the intensity of the flavor. They made no study of the physical or chemical properties of the milk. Later, the same authors (25) found that the addition of 0.1 g./l. of KI would inhibit. A study of the passage of KI from the feed to the milk would be interesting. Recently, Krukovsky and Guthrie (130) concluded that ascorbic acid is a link in the chain forming the flavor. They based this conclusion on the observation that the oxidation of ascorbic acid by H_2O_2 inhibits development and milk so treated can be made to develop the flavor by adding ascorbic acid. Later, the same authors concluded that it is the "pressures" of ascorbic and dehydroascorbic acid which control the development of the flavor. Greenbank (85) explains these reactions in a slightly different manner. He concludes, according to his intermediate oxidation product theory, that when all the ascorbic acid is destroyed the E_h is high enough to produce a completely oxidized form of the causative agent which has no flavor. The addition of more ascorbic acid to the milk lowers the E_h so that the intermediate or flavored compound may form (83). The correlation of "pressures" of ascorbic-dehydroascorbic acid is another way of expressing the ratio of the reduced to the oxidized form. This ratio is the basis for E_h . These data may be used to confirm Greenbank's (82, 83) conclusion that the development of the flavor is related to the change in E_h . Guthrie *et al.* (92) found a general relationship

between factors which accelerates the rate of oxidation of ascorbic acid and development of the flavor.

Riboflavin (lactochrome) vitamin B₂. The green pigment in whole milk is concerned in a number of ways with biological oxidations, according to Ball (13). When combined with a specific protein, riboflavin becomes an enzyme. Such compounds are called flavoproteins. One of these is Schardinger's enzyme. It is not known whether it plays any part in the development of the flavor, but one interesting fact is that this enzyme is concentrated on the surface of the fat particles. Separating milk concentrates the flavoprotein in the cream and churning the cream concentrates it in the buttermilk (98, 101). It may be significant that flavoprotein is found in the following increasing order: Milk < cream < buttermilk, which is the order of increasing susceptibility to development of the flavor. The acceleration of the photochemical oxidation of ascorbic acid by riboflavin is discussed under "Irradiation."

Proteins, lactose, and salts. These constituents do not seem to be concerned in the development of the flavor, because they are stable towards oxidation under conditions most favorable to the development of the flavor (83).

BIOLOGICAL FACTORS

Biological factors are important because they influence the properties of the milk. In studying the biological factors, it is important that changes in constituents and properties be observed at the same time. Many conclusions found in the literature are not of much value because variables other than the one studied were not controlled.

Breed. The relationship of breed to the development of the flavor has been studied because certain breeds seem to synthesize carotene from their feed more readily than others and thus produce a more highly colored milk. Color has been thought to be related to the development of the flavor. Whitnah *et al.* (228) found that all the samples in which the flavor developed were below the breed average in intensity of color. However, they found samples low in color that did not develop the flavor. They found also that the average vitamin C content of milk from different breeds increased in the following order: Holstein, Ayrshire, Guernsey and Jersey, while the spontaneous development of the flavor decreased in the same order (228). If ascorbic acid acts as a flavor inhibitor, the order of susceptibility given is correct.

Bacterial count. It has been known for some time that milk of low bacterial count is more susceptible than milk of high count, provided all other conditions are the same or similar. It has been postulated that the bacteria utilize the dissolved oxygen and form metabolic products which lower the E_h or are inhibitors. The growth of bacteria is one of the factors which inhibits development of the flavor at high storage temperatures. Thurston and Olson (209) found that milk stored at 38° F. had little bacterial growth and developed the flavor, while a sample of the same milk stored at 52° F. had considerable bacterial growth and did not develop the flavor. Roland *et al.* (178) found that the bacterial counts were generally lower in milk which developed the flavor than in

milk which did not develop it. Greenbank (83) found that bacterial growth inhibits the flavor development and decreases the E_h at the same time. Other workers have obtained similar results (1, 57, 202, 203). A number of workers have concluded that bacterial growth inhibits the development of the flavor (3, 53, 212). However, the evidence indicates that the number of bacteria required to exhaust the oxygen or reduce it to a concentration low enough to inhibit would be sufficient to produce serious bacterial defects (46, 64, 134).

Period of lactation. Brueckner and Guthrie (33) were among the first to study the relation of this factor to the development of the flavor, but they were unable to find any correlation. Rasmussen *et al.* (165) found that the ascorbic acid content of milk is relatively high during the early stages of lactation and decreases to a minimum in about 2 months but rises to a maximum during the latter stages. If ascorbic acid content is the controlling factor (130), milk from the middle of the lactation period should be the most susceptible and late lactation the least susceptible milk. This is contrary to the evidence of Corbett and Tracy (39), who found that milk from the first part of the lactation period is most susceptible, especially in the case of heifers.

Feed. Brueckner and Guthrie (33) were among the first to show that when cows are fed green feed they produce more stable milk than when they are fed dry feeds. This same conclusion has been made by a number of workers (27, 42, 53, 121, 204, 206). However, Hening and Dahlberg (104) found that feeding below the Morrison standard did not affect the flavor. They also found that feeding mangels or beet pulp had no effect on the flavor (103). Majer (137) found fresh "alp" hay in the ration inhibited development. Stebnitz and Sommer (198) found that when cows receive grass as a part of the ration the butterfat becomes more unsaturated and therefore more susceptible to oxidation, and the milk becomes less susceptible to development of the flavor. These data may be used to indicate that the fat is not responsible or that the liquid phase plays some part in controlling development of the flavor. Garrett (66) reports that green grasses or legumes preserved as silage or artificially dehydrated hay are especially desirable in preventing development of the flavor. Bartlett *et al.* (14) found molasses silage of immature grasses or legumes excellent in producing milk highly resistant to the flavor development. Babock and Haller (12) found that feeding different silages had no effect on the copper tolerances of the milk produced.

Dry feeds. The effect of feeds is dependent to a great extent on the preservation of their nutritive value in drying. Anderson (5) concludes that feeding good machine cured alfalfa changes poor milk to good milk and that poor alfalfa will do the reverse. Brown, *et al.* (30) found that feeding high quality alfalfa with alfalfa meal greatly reduced or eliminated metal induced flavor. Feeding of brown leafy alfalfa did not increase the tendency to develop the flavor. Dahle and Carson (47), on the other hand, found that feeding alfalfa hay produces milk more susceptible to the flavor than milk from cows fed on other roughages. Brown *et al.* (27) found that dry feeding increases the susceptibility and green feeding reduces it. Guthrie and Brueckner (90) found that dry feeds are not

the sole cause of the flavor, because milk from separate quarters of the udder developed different flavor intensities.

Supplements. The feeding of supplements is an attempt to supply essentials that are absent from poor feed. In correlating the feeding of these supplements, it is essential that a study of all the changes in the milk be made, otherwise, the conclusions may be misleading. The feeding of carrots is beneficial in preventing development of the flavor (9). Corbett and Tracy (38) fed cocoanut and corn oil and found that the iodine value of the milkfat was increased markedly, but there was only a slight change in susceptibility. Brown *et al.* (28) fed 1 lb. of cocoanut oil per day. The iodine value of the milkfat increased slightly and there was also a slight increase in susceptibility. One lb. of soybean oil increased the iodine value greatly and increased susceptibility to copper induced flavor. Prewitt and Parfitt (162) fed 14 cows ground soybeans, soybean oil, linseed meal, dried brewers yeast, and none of the milk from these cows developed the flavor spontaneously. However, milk from cows which were fed soybean oil or meal was least susceptible to metal-induced flavor. Brown *et al.* (27) fed 1 quart of lemon or tomato juice per animal per day and reduced susceptibility. The same authors found that feeding 0.5 g. of ascorbic acid per day reduces the tendency to develop the flavor. Brown *et al.* (29) fed 5 g. of KI per day and noted a marked decrease in the ascorbic acid content of the milk but no increase in metal-induced susceptibility. No study was made of changes in the other properties of the milk. Later, Brown and Olsen (25) found that 0.1 per cent KI added to milk would prevent development of the flavor.

Anderson *et al.* (9) found that feeding 8 lb. of carrots per day in the ration was more effective in inhibiting development of the flavor than the addition of 500,000 units of U.S.P. carotene. Whitnah *et al.* (229, 230) and Beck *et al.* (15) found a carotene supplement quickly corrected the tendency for the flavor to develop spontaneously. Brown *et al.* (24) found that a carotene supplement rendered the milk more resistant to the metal-induced flavor. Martin *et al.* (143) fed 1/3 g. of carotene per day for 15 days and increased the color of the milk 60 per cent, with a decrease in the flavor intensity. Brown *et al.* (29) studied the effect of ascorbic acid and carotene as supplements on the development of the flavor but did not study the effect of carotene in the ration on the ascorbic acid content of the milk.

Seasonal variations. Mattick (144) was one of the first to report a seasonal variation in the production of the flavor. He found that the flavor appeared in autumn, winter and spring but never in summer. More recently, the variation in susceptibility between winter and summer milk has been observed by many workers (5, 27, 33, 42, 90, 163, 204, 211, 217, 223). The greater susceptibility in winter, as observed by most workers, seems reasonable as shown by the discussion on green feed and its effect on the flavor. Anderson and coworkers (3, 4) found that the titratable acidity of milk is higher in winter than summer and concluded that there was a correlation between titratable acidity and development of the flavor.

THE EFFECT OF PROCESSING

Processing may change the properties of milk so as to inhibit or promote development of the flavor. In the discussion, an attempt will be made to point out changes in the properties of the milk which may influence the development of the flavor.

Heat treatment. Most workers conclude that pasteurization has little effect on the development of the flavor unless metallic contamination occurs. However, Dahle (43, 42) reported that heating milk to 145° F. for 30 minutes intensified the flavor. Gjessing and Trout (71) concluded that the ascorbic acid was less stable in milk pasteurized by holder methods than in milk pasteurized by using higher temperatures, especially when copper is present. Woessner *et al.* (231) concluded that 20 per cent of the ascorbic acid was destroyed in the holder methods of pasteurization. They also concluded that a temperature of 167° to 185° F. for 15 seconds is required to stabilize ascorbic acid. The heat treatment necessary to inhibit development of the flavor is, according to Kende (121), 185° F. for 5 minutes, Sharp (191), 170° F. for 10 minutes, Dahle and Palmer (53), heating to 170° F. Kende (121) and Sharp (185) concluded that heating kills an enzyme which promotes the oxidation. Greenbank (83) found that heat treatment reduces the E_h and attributes retardation to a reduced E_h . This has been confirmed (78, 119). Gould and Sommer (78) and Gould (75, 76) have shown that sulphydryl compounds, which are of a reducing nature, are formed when milk is heated to temperatures above pasteurization and these compounds produce the cooked flavor.

Storage temperature. The effect of storage temperature on the development of the flavor is one of the factors which supports the theory that the reaction is a mild chemical oxidation and not enzymatic. The intensity of the flavor increases as the storage temperature decreases. This correlation is paralleled in many chemical oxidations and is contrary to the effect of variations in temperature on enzymatic reactions. Lowering the storage temperature should make the oxidizing conditions milder but has been observed to increase the intensity of the flavor (83). Tracy (210) was probably the first to observe that the flavor developed more rapidly at 4° C. than at 20° C. Greenbank (83) verified these results. Bell (15) observed that concentrated milk held at -17° C. became oxidized more rapidly than at -7° C., and also that the intensity at -7° C. finally decreased while that at -17° C. remained the same. However, Thurston and Olson (209) noticed an oxidized flavor in milk held at 38° F. which showed little bacterial growth. The same sample held at 58° F. showed considerable growth, and the flavor was not detected. Tracy *et al.* (212) found that milk incubated from 1 to 6 hours at 68° F. or at 90° F. and subsequently stored at 40° F. is less likely to develop the flavor than milk stored immediately at 40° F. A number of investigators have worked on the development of the flavor in cream (58, 149, 150, 184, 195). The inhibition at higher storage temperature has been attributed to a number of factors. Kende (121), Tracy *et al.* (212) and Greenbank (83) attribute the inhibition to a lowering of the E_h by bacterial growth. Tracy

et al. (212) also conclude that the bacteria use up the dissolved oxygen, but Sharp *et al.* (190) conclude that to do this there would be a deterioration in flavor due to excessive bacterial growth.

THE EFFECT OF METALLIC CONTAMINATION

Copper. Copper is a normal constituent of milk. However, contamination by this metal has been studied for years (11, 105, 126, 136, 172). Rogers *et al.* (177) probably were the first to conclude that copper contamination caused a more intense tallowy flavor in butter than did iron. Copper is an ideal catalyst for the development of this flavor, because it is a relatively mild oxidizing agent and high concentrations will not inhibit development of the flavor (83). Golding and Feilman (73) were probably the first to report the development of a metallic flavor in milk passed over a detinned cooler. Hunziker and Hosman (114) were among the first to point out that copper contamination produces a more intense flavor in milk than does contamination by iron. The mechanism concerned in the action of copper has been studied by a number of investigators. Osborne and Leavenworth (158) and Vandeveld (221) found that copper combines with protein more as an absorption complex than as a chemical compound. Olson and Brown (157) found that copper combines with the ascorbic acid anion and thereby promotes oxidation; Brown *et al.* (26) found that the intensity of the flavor was greater when copper was added after rather than before pasteurization. This has been confirmed by Greenbank (83).

Iron. This metal, like copper, is a normal constituent of milk. Contamination by iron is not as detrimental as contamination by copper. This has been observed by many workers (26, 83, 136). It has been shown that ferrous iron promotes the development of the flavor but requires a much higher concentration than does copper (83, 201). Ferric iron is less effective than ferrous iron and may even inhibit the flavor (83). Samples containing ferric iron were found to possess the flavor after storage for 24 hours but not for 48 hours (83). Hartman *et al.* (100) found ferrous iron lowered the E_h and did not cause as intense a flavor as copper. This has been confirmed by Swanson and Sommer (201). The effect of copper and iron on the development of the flavor has been studied by a number of workers (36, 41, 54, 55, 70, 72, 74, 80, 88, 93, 128, 142, 147, 148, 150, 171, 194, 195, 222, 225).

Other metals. Besides those already discussed, aluminum, lead, nickel, tin and zinc are the metals used in pure form or as alloys in dairy equipment. Nickel has been studied extensively by a large number of workers (26, 36, 41, 54, 55, 59, 63, 68, 70, 74, 93, 132, 146, 171, 215, 226, 227). Guthrie *et al.* (94) report the development of the flavor by nickel contamination in one case. Whitefield *et al.* (227) report a metallic flavor was caused by contamination by nickel. Fink and Rohrman (63) found that during pasteurization nickel may replace copper that is in solution and render the milk less likely to develop the flavor. Aluminum has been found by many workers to be without effect (63, 68, 94, 109, 112, 129, 171, 225, 227). Allegheny metal, chromium nickel steel and stainless steel are not corroded by milk and do not promote development of the flavor (109, 112, 113, 122, 164, 225, 226, 227).

Manganese, lead and zinc do not influence the oxidation of ascorbic acid and do not affect the flavor.

Irradiation. The exposure of milk and dairy products to light is a form of irradiation. The effect of light on the flavor of dairy products has been studied since 1890 (95). A large number of workers have observed the effect of sunlight on milk (36, 65, 140, 141, 145, 163, 206). The effect seems to be dependent upon the intensity, wave length and time of exposure (83). Hand *et al.* (97) have verified Hopkin's work (108) which indicated that the riboflavin of milk catalyzes the photochemical oxidation of ascorbic acid and itself is changed in the reaction to lumichrome. After the destruction of the riboflavin, the ascorbic acid is stable to the action of light (97, 108, 124). The addition of more riboflavin restores the reactivity (98). Burr (35) probably was the first to observe that exposure to light hastened the deterioration of milk and that dark bottles would prevent deterioration. Hammer and Cordes (96) confirmed Burr's work and added that copper and iron hastened the development of the flavor. Sharp *et al.* (187) found that part of the vitamin C is destroyed by sunlight if oxygen is present, but if the milk is deaerated the vitamin C is not destroyed. They also found that irradiation to produce vitamin D in milk decreases the vitamin C content from 3.4 to 1.7, 3.8 to 1, and 11.0 to 5.5 mg./l. Trout and Gjessing (217) found a slight destruction of vitamin C by irradiation. Guthrie *et al.* (92) found that paper bottles decrease the effect of sunlight on the oxidation of ascorbic acid and on the development of the flavor. On the contrary, Doan and Meyers (56) found that the flavor is more intense in milk stored in paper than in milk stored in glass bottles, but paper bottles did protect against development of burnt flavors. It would appear as if the difference here is one of light transmission or intensity of the incident light and that the bottles used by Doan and Meyers transmitted some of the shorter wave lengths which cause burnt flavor (83). Marquardt (139) found that 20 to 60 minutes in sunlight caused the flavor to develop in 24 hours and 2 hours caused a bleaching effect. According to Greenbank (83), light may inhibit, promote or have no effect on the development of the flavor, depending on the metallic contamination of the milk and the intensity of irradiation.

Dissolved gases. Fresh whole milk drawn without gaseous contamination contains dissolved gases of which 81.5 per cent is CO₂, 2.42 per cent O₂ and 16.54 per cent N₂ (168). Milking increases the O₂ content to 13.18 per cent. Guthrie (89) found that milk direct from the udder contains from 0 to 11 mg./l. of O₂. According to Sharp *et al.* (190), hand milking introduces 5.8 and machine milking 4.7 mg./l. of oxygen.

The effect of air on the deterioration of milk and dairy products has been studied for years (95, 117, 118).

Hartman and Garrett (99) found that the ratio of oxygen consumed to ascorbic acid oxidized increases progressively as the oxidative reaction proceeds. After the ascorbic acid is oxidized, there is a further consumption of O₂ presumably by the oxidation of the fatty substances. Greenbank (83) observed that aeration, and Sharp *et al.* (190) that deaeration inhibited development of

the flavor. These reactions are discussed under the heading "Prevention." Thurston *et al.* (208) and Greenbank (80) found that prolonged stirring inhibits the flavor. This may be assumed to be a form of aeration. Deoxygenation by bacteria is discussed under "bacterial count."

Homogenization. Tracy *et al.* (212) were probably the first workers to observe that homogenization retards the development of the flavor. More recently, Thurston *et al.* (208), Dahle (45) and Ross (180) observed the same effect. Trout and Gould (218) report that homogenization does not retard development when the copper contamination is too high. Larson *et al.* (133) confirmed the previous work but found no relationship between extent of inhibition and the changes in E_h . While there is not a direct correlation at every point between the flavor and E_h , the work does show that the E_h of the homogenized samples maintains a relatively high potential for at least a week, while the unhomogenized samples show a marked drop in potential after the first day (133).

METHODS OF PREVENTION

Aeration and deaeration. The results of a number of workers seem to prove quite conclusively that the development of the flavor may be inhibited by either aeration or deaeration (53, 80, 190). Greenbank (80) was able to inhibit the development of the flavor by aeration or addition of hydrogen peroxide. The same author was able to increase copper tolerance by aeration. Prolonged agitation, which is a form of aeration, has been found by Thurston *et al.* (208) to inhibit development of the flavor. Dahle and Palmer (53) were probably the first to conclude that removal of oxygen dissolved in the milk would prevent development of the flavor. More recently, this conclusion has been confirmed by other workers (91, 92, 97, 188, 189, 190). Sharp *et al.* (187) have developed a commercial method of deaeration which protects the milk for 7 days when contaminated with 0.1 mg./l. of copper. Brown *et al.* (32) found that the flavor developed most rapidly in vacuum capped bottles. Greenbank (83) found that deaeration would not protect against flavor development when relatively high concentrations of copper were present.

Elimination of metallic contamination. Much of the oxidized flavor in milk would be eliminated if metallic contamination did not occur. Roadhouse (175) found that passing 5 gallons of hot milk through a bronze pump caused the development of the flavor and loss of 2 points in score. Nickel in the equipment may replace copper in solution; according to Fink and Rohrman (63). The use of stainless steel and enameled equipment practically eliminates the possibility of the flavor developing from contamination.

Segregation. A simple method of preventing the development of the flavor is to eliminate individual samples of milk which are "spontaneous" or develop the flavor with low metallic contamination. Spontaneous milk may be detected by storing individual samples at 4.0° F. Susceptible milk of low copper tolerance may be detected by the increase in E_h after the addition of copper, according to Greenbank (81).

Antioxidants. Although the addition of antioxidants to milk is prohibited

by law, a number of workers have studied the action of these compounds in milk. Ritter and Christen (173) used a dried culture of bacteria which Kertesz (123, 124) called "*Reductobacterium frigidum neutrale*" and inhibited the development of the flavor. The authors isolated 5 to 7 per cent hydroquinone from the dried culture. Bird *et al.* (17) found that the higher the content of iron the less tendency for the flavor to develop. They believed that the iron combines in a ferrous form with the protein to serve as an antioxidant. Anderson (8) reported the use of pancreatic enzyme prevented development of the flavor whether metal contaminated or not. Russell and Dahle (182) found that concentrated or dried milk added to fluid milk acted as an antioxidant. Dried milk is more effective than concentrated milk. Ritter (169) found hydroquinone, metol and ascorbic acid inhibited development of the flavor. The effect of ascorbic acid and hydroquinone has been confirmed by Chilson (37), Dahle and Palmer (53) and Greenbank (83). Ascorbic acid sometimes is considered an antioxidant. While it does retard development, there remains the question, according to Greenbank (84), whether this action is that of a reducing agent to lower the E_h or to supply protons to regenerate the natural antioxidants in milk. Oat flour, known as Avenex, has been found by many workers to have antioxygenic properties (32, 34, 45, 51, 52, 153, 160).

Condensing and drying. Corbett and Tracy (39) report that concentrating milk to double the solids content prevented the development of the flavor in the condensed and reconstituted milk. The addition of concentrated or dried milk before pasteurization was more beneficial than addition after pasteurization. The addition of 0.2 per cent solids-not-fat had a noticeable effect on the flavor. Dahle and Folkers (49) and Ross (181) found that ice cream containing dry skim milk did not develop the flavor, but the flavor developed when the ice cream contained condensed skim milk. The inhibiting action of these products is probably due to the high heat treatment they received. Krukovsky and Guthrie (130), in a study of ascorbic acid as a key factor in the development of the flavor, concluded that complete oxidation of the ascorbic acid will inhibit development of the flavor. Previously, Greenbank (83) found that the addition of hydrogen peroxide would inhibit the flavor development but did not relate this action to the destruction of ascorbic acid. He concluded that the inhibition was the result of a more complete oxidation of the precursor, presumably to a tasteless form.

THE OXIDIZED FLAVOR IN DAIRY PRODUCTS

Cream. According to many workers, cream is more susceptible to the flavor development than milk. The fact that the phospholipid content of cream is greater than that of milk may be significant. Cream, which was susceptible to the flavor when incubated according to the method of Tracy *et al.* (212), scored 3.5 points higher than a sample of the same cream stored at 40° F. without incubation. Kooper (127) observed that cream held in rusty cans developed a metallic flavor. Another worker (11) confirmed this. A number of investigators have found that it is not so much the exact temperature, provided it is low,

as the metallic contamination in stored cream which induces the flavor development (58, 149, 150, 151, 184, 200).

Condensed milk. The development of the flavor is not as common in condensed milk as in milk or cream, but it has been observed by a number of workers (57, 69, 111, 167). Sommer and Gebhardt (197) report that the flavor of evaporated milk is destroyed in proportion to the copper content; Corbett and Tracy (40) reported that condensing to twice the solids content prevented development of the flavor in the condensed and the reconstituted milk. They attribute inhibition to the liberation of antioxidants derived from the proteins. The high heat treatment should inhibit the development of the flavor, according to other workers (78, 83, 119).

Ice cream. This product is probably less susceptible to the development of the flavor than cream. Strawberry ice cream seems to be very susceptible. However, Dahle *et al.* (47, 49) found pineapple ice cream just as susceptible. Dahle and Folkers (50) and Tracy *et al.* (213, 214) report that increased amounts of berries delayed the onset. They also found that soaking the berries in the mix retarded the development. Heating the berries used to 150°, 175° and 200° F. or autoclaving the berries at 15 lb. pressure for 15 minutes decreased the flavor in the order given but did not eliminate it. Dahle *et al.* (50) were unable to eliminate the flavor by heating berries for 1 hour at 180° F. Mudge and Tucker (151) reported that aeration of the berries for a considerable time resulted in a stale and unclean flavored ice cream. The presence of metallic salts in the berries has been given as a reason for the development of the flavor. Ross (181) reports that iron is a factor, and Iverson (115, 116) reports that it is not a factor in the development of the flavor. The latter postulates that ferrous iron combines with protein and acts as an antioxidant. Mack and Fellers (136) observed that the acidity of the berries induced flavor development. Tracy *et al.* (214) observed that the citric acid content had no effect. The latter authors found that apples, apricots, lemons, oranges, pineapples and peaches promote the flavor when in concentrations of 3 to 5 per cent. The work of Dahle and Josephson (51) indicates that vanilla ice cream will develop the flavor but not as readily as strawberry. This difference may be due to the vanillin which has antioxygenic properties. Schricker (193) found that the development of the flavor occurs with increasing intensity in the following order: chocolate, vanilla and strawberry. Chocolate is probably protected by the tannins in the chocolate which are antioxidants and as previously stated, the vanilla by vanillin. Dahle and Folkers (48) and Ross (181) found that dry milk has a tendency to inhibit but that in most samples containing condensed milk the flavor developed, probably because dry milk gets the higher heat treatment and contains more reducing substances. Ross (181) observed that condensed milk contained more copper and developed the flavor sooner; Dahle and Folkers (50) found that the flavor in milk or skim milk has little effect on the good flavor of the ice cream. Bird *et al.* (17) found more copper in condensed skim milk than in the skim milk powder; the ice cream made with the powder developed a stronger flavor than ice cream in which condensed

milk was used. Dahle and Josephson (51, 52) report that 0.3 per cent of Avenex was not quite sufficient to inhibit completely the development of the flavor in strawberry ice cream. Mueller and Mack (152) found 0.25 per cent sufficient to delay development of the flavor but 0.50 per cent was still more effective. Weckel (224) suggested the use of not more than 0.3 per cent for vanilla and 0.50 per cent for strawberry ice cream. Mack and Tracy (135) and Burke and Newman (34) and also Brown (31) found that 0.5 per cent of Avenex was sufficient to insure a fresh flavor.

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THE KEEPING QUALITY, SOLUBILITY, AND DENSITY OF POWDERED WHOLE MILK IN RELATION TO SOME VARIATIONS IN THE MANUFACTURING PROCESS.

II. SOLUBILITY AND DENSITY¹

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In judging the quality of whole milk powder, first consideration should be given to palatability. Of almost equal importance for proper consumer acceptance is the solubility of the powder and the ease with which it may be reconstituted.

REVIEW OF LITERATURE

Solubility. There are many reports which show that the temperature-time relationship of preheating of the milk has a direct relationship to the solubility of the resulting powder. However, it should be borne in mind that solubility is relative and depends upon the solubility test used.

Crossley and Johnson (4) concluded that the solubility of milk powder was least impaired when the preheating temperature did not exceed 159° F. for 20 seconds. For temperatures of between 150 and 163° F. for 20 seconds, followed by a 3 to 5 minute holding period at a slightly lower temperature, the mean solubilities found were nearly a constant value only just below that obtained at 159° F. Even at 167° F. the reduction in solubility was not of commercial significance. Hollender and Tracy (6) compared the solubility indices of powders made from whole milk preheated at 150, 170 and 190° F. for 30 minutes and found that the least soluble powders were those made from the milk preheated at 190° F. However, Crossley (3), by using the short-time preheating method, found little loss in solubility in powder made from milk preheated at 190° F. as compared to that in powder made from milk preheated at 165° F., and the very small loss of solubility appeared to be more than offset by the increased keeping quality with respect to flavor.

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Wright (20), in studying milks of concentrations varying from 20 to 50 per cent total solids and preheated at 194, 203 and 212° F., found that for a given temperature, the higher the concentration of the milk the more readily was the protein rendered insoluble. He reported that when milk powder containing 2.5 per cent moisture was exposed to temperature-time relationships ranging from 212° F. for 10 hours to 282° F. for 40 seconds, 50 per cent of the casein was rendered insoluble. In another experiment, Howat and Wright (7) heated spray-dried whole milk powder for 6 hours at 221 to 230° F. Sixty per cent of the protein was insoluble when the powder was reconstituted at 68° F. Crossley and Johnson (4) found that prolonged exposure of milk powder to hot air currents, even at 194° F. or less (in bag dust collectors), resulted in significantly lowered solubility.

In 1931, Lampitt and Bushill (10) reported that the decrease in solubility of powders of comparable moisture content (less than 6 per cent moisture) was almost negligible when stored at room temperature and very much slower than at 86° F. Lea and Smith (12) concluded that changes in solubility due to storage at ordinary temperatures for a number of years would be exceedingly slight with gas-packed powders at reasonably low moisture contents. Lea *et al.* (11) found that a moisture content of 2.2 per cent had no appreciable effect on solubility.

Hollender and Tracy (6) stated that when powders were stored at temperatures below 68° F., variations in the moisture content between 2.32 and 5.39 per cent had no significant effect on the solubility of the various powders studied. They observed that those samples which discolored during storage became less soluble, that 5 per cent moisture content is about the critical point and upper limit for samples to be stored at room temperature without discoloration, and that at temperatures of 68° F. or above discoloration takes place regardless of the moisture content. They concluded that conditions favorable for brown discoloration and decrease in solubility of milk powders were preheating the milk at 190° F. for 30 minutes, a moisture content of 5 per cent or higher and storing the powder at temperatures of 68° F. or higher.

Wilster *et al.* (19) listed small particle size, small, uniform-sized fat globules and care not to overheat the powder in the drying chamber as favorable factors for good solubility of whole milk powder.

Density and particle size. Webb and Hufnagel (18) found that the density of milk powder could be increased by increasing the degree of preconcentration. Mook (15) described a patented method in which the milk was preheated at 160 to 170° F., condensed to 35 to 40 per cent total solids, and then superheated at not less than 170° F. and preferably at 180 to 210° F. until slight coagulation occurred. The milk powder from this milk was reported to be of greater density and to reconstitute to a fluid with four to eight times the viscosity of reconstituted milk from powders produced by the usual procedures.

Miyawaki (14) found that milk powder made from milk sprayed without preheat treatment had more and larger air cells than the powder made from

preheated milk. Coulter and Jenness (2) reported that the removal of foam from the condensed milk prior to spraying reduced the amount of entrapped air in the powder particles and that, on the other hand, whipping air into the precondensed milk resulted in greater numbers of air cells and air cell clusters in the powder particles. Studying the effect of preheat treatment of milk upon the density of the milk powder, Stamberg and Bailey (16) found that the densest powder was made from milk receiving the most severe heat treatment. The preheat treatments studied were: 150 and 200° F., and 150 and 200° F., followed by superheating. The holding time during preheating was constant for all samples, but it was not definitely recorded.

Hunziker (8) stated, "It has long been observed that an increase in the concentration of the fresh milk is accompanied by an increase in the particle size of the resulting spray powder." According to Hetrick and Tracy (5) and Hollender and Tracy (6), large-size powder particles were obtained by using a low homogenization pressure, low spray pressure and low spray temperature.

EXPERIMENTAL PROCEDURE

The manufacturing and storage procedures used were given in an earlier publication (13).

Solubility test. The solubility indices of the powders were determined by the method of Cone and Ashworth (1) at intervals of 24 hours and 1, 2 and 6 months following manufacture.

It was noted that powder made from the 40 per cent total solids concentrate had a higher solubility index than the powder made from the 20 per cent total solids concentrate. A portion of the original unconcentrated milk for powders no. 108 and 109 was homogenized and subjected to the solubility test. The homogenized concentrated milk from which powders 94 to 109, inclusive, were made also was subjected to the solubility test and the results compared to the solubility index of the fresh powder. In addition, the homogenized concentrates from which powders no. 108 and 109 were made were examined microscopically to determine if any differences in homogenizing efficiency existed due to differences of concentration of the milk. The per cent of fat globules greater than 5 μ in diameter in given fields under the oil immersion objective of the microscope was estimated by Cone and Ashworth (1) by use of a previously calibrated Whipple eyepiece.

Density and particle size. The apparent density was determined on the powders stored at 45° F. for 6 to 7 months by using the following procedure: Light mineral oil (no. 1) manufactured by the Standard Oil Company was employed as the displacing medium. Eight per cent Babcock milk test bottles were found to serve excellently as improvised pycnometers (17). The Babcock test bottles were carefully calibrated at 25° C. with freshly-boiled distilled water at three different levels between the 6.5 and the 8.0 per cent marks on the necks of the bottles. The level of the bottom of the meniscus was estimated to 0.1 of the 0.1 per cent divisions. The density of the oil was determined by use of Hubbard-Carmick specific gravity bottles and by use of the above-mentioned calibrated

Babcock test bottles. In both cases the average value obtained was 0.8407 with a standard deviation of ± 0.00001 . However, the figure 0.841 was used in the density determinations of the powder since the reading of the meniscus of the oil level to 0.1 of a 0.1 per cent division was an estimation to 0.002 ml. From 7 to 11 g. of the powder were introduced into each calibrated Babcock test bottle by use of a 2-inch glass funnel from which the stem had been removed. The apex of the funnel was placed within the flared opening of the test bottle and the powder in the funnel was vibrated into the bottle by use of a "vibro" glass marking tool. Mineral oil then was added so as to fill the bulb of the test bottle about three-fourths full. The powder and oil were mixed thoroughly by vigorous shaking and then were subjected to 20 inches of vacuum for 20 minutes to remove interstitial and adsorbed air. The above conditions for the removal of interstitial and adsorbed air were found to give constant results, whereas a lesser period of time resulted in the appearance of free air bubbles and a period of 30 minutes did not change the results obtained. Upon removal from the vacuum, the oil level was raised with additional oil to approximately the 7 per cent mark. The samples then were tempered in a $25 \pm 0.1^\circ$ C. water bath. At the end of 30 minutes the levels of the bottom of the menisci were recorded, the bottles were wiped dry and weighed, and the densities calculated.

The solubility effect of the mineral oil on the constituents of the milk powder was checked by determining the specific gravity of mineral oil filtered from completed milk powder density determinations. The oil had been in contact with the whole milk powder 2.25 hours at the time filtration was begun; filtration required an additional 2.75 hours. Triplicate determinations on the specific gravity of the oil gave 0.8409, 0.8409 and 0.8410 as the specific gravity.

Centrifugation also was tried as a means of removing the interstitial and adsorbed air. Although the results obtained were approximately the same as when vacuumization was used, the appearance of very fine particles in the oil meniscus precluded accurate reading of the oil level in the stem of the test bottles.

The sizes of the dry milk particles were determined by examining under the oil immersion objective a mineral oil suspension of the powder placed between a glass cover slip and a glass slide. The particle sizes were estimated by use of a previously calibrated Whipple eyepiece with a grid division of 2.5μ . The number of particles measured per batch varied from 1000 to 5900 particles.

RESULTS AND DISCUSSIONS

Solubility. All solubility indices given in table 1 are the averages of three determinations. The degree of preconcentration has a very distinct effect on the solubility of milk powder. In every comparison of paired milk powders, the powder made from the 40 per cent total solids concentrate had a higher initial solubility and maintained a higher solubility during storage than did the powder made from 20 per cent total solids concentrate. Powders made from either 20 or 40 per cent total solids concentrate showed little if any decrease in solubility when stored at 45° F. for 6 months. However, when stored at 100° F. for 6

months, powders made from 20 per cent total solids concentrates showed a distinct lowering of solubility indices. This decrease was least for the powders made from milk preheated at 160° F. for 30 minutes.

The powders made from the 40 per cent total solids concentrate, except those made from milk preheated at 180° F. for 10 minutes, retained to a remarkable degree their initial solubility when stored at 100° F. for 6 months. The powder made from milk preheated at 180° F. for 10 minutes decreased rapidly in solubility. It is suggested that the denaturing of the casein initiated by the high preheat treatment lowers the solubility of milk powders during storage as is indicated also in the data presented by Hollender and Tracy (6). They found that powders made from milk preheated at 150 and 170° F. for 30 minutes did not de-

TABLE 1
Solubility indices of whole milk powders

Preheat treatment	Milk conc., average	No. of samples	Powder, ave. % moisture	Average solubility index after storage for			
				1 day	1 mo.	2 mo.	6 mo.
(% T.S.)							
Storage at 45° F.							
160° F.	20.2	6	2.7	97.4	97.3	97.4	97.3
30 min.	38.1	6	2.2	98.8	98.5	98.5	98.5
170° F.	21.9	4	2.2	98.1	98.5	98.4	98.3
10 min.	40.7	4	2.0	99.3	99.3	99.3	99.3
170° F.	21.0	6	2.3	97.8	98.5	98.4	98.3
30 min.	40.9	6	2.1	99.2	99.4	99.4	99.4
180° F.	22.8	6	2.3	98.4	98.9	99.0	99.0
10 min.	40.3	6	2.0	99.1	99.4	99.4	99.3
Storage at 100° F.							
160° F.	20.2	6	2.7	97.4	97.2	97.3	96.9
30 min.	38.1	6	2.2	98.8	98.7	98.8	98.5
170° F.	21.9	4	2.2	98.1	97.5	97.6	96.7
10 min.	40.7	4	2.0	99.3	99.3	99.3	99.1
170° F.	21.0	6	2.3	97.8	98.1	98.1	96.9
30 min.	40.9	6	2.1	99.2	99.4	99.4	99.1
180° F.	22.8	6	2.3	98.4	98.1	98.2	96.9
10 min.	40.3	6	2.0	99.1	99.2	99.1	97.4

crease in solubility as did powders made from milk preheated at 190° F. for 30 minutes when stored at room temperature for 67 days.

The preheat treatment of the milk, as used in this experiment, appears to affect the initial solubility of the milk powder only to a very slight degree. The initial solubility of the milk powder made from milk preheated at 160° F. for 30 minutes was slightly lower than that of milk powder preheated at the other temperatures. The initial solubilities of the powders made from milk preheated at 170 and 180° F. for 10 minutes and at 170° F. for 30 minutes were about equal when the powders were made from the 40 per cent concentrate. These results are not exactly comparable to those reported by others (6), who showed that the powder made from milk preheated at 190° F. for 30 minutes was less soluble than the powder made from milk preheated at 150 and 170° F.

TABLE 2

Comparison of the solubility index of homogenized whole milk, homogenized concentrated milk and the powder made from the concentrated milk

Powder no.	Milk and conc. milk	% of fat globules over 5 μ in diam.	Solubility index	
			Milk	Powder
	(% T.S.)			
	12.8 ^a	10	97.8	
108	22.6	5	98.9	97.6
109	38.6	less than 1	99.3	97.9

^a Original milk from which concentrates for powders no. 108 and 109 were made.

for 30 minutes. These differences probably are due to the shorter period of exposure and the lower maximum temperature employed and possibly due to the differences in method employed to determine solubility.

Efficiency of homogenization. It appeared from microscopic examination that the more highly concentrated milk is homogenized more efficiently. The per cent of fat globules 5 μ or over in size progressively decreased as the pre-concentration increased. The solubility test (1) when applied to these milks indicated a direct relationship between high solubility indices and efficient homogenization. Under the conditions of the solubility test employed, it is impossible to have a solubility index of 100 per cent. Thus, the unconcentrated, unhomogenized sample shows the lowest solubility index because at least a part of the cream layer is retained as insoluble material (table 2).

In table 3 is shown a further comparison of the solubility indices of concen-

TABLE 3

Comparison of the solubility of homogenized concentrated milk and the powder made from it

Powder no. ^a	Preheat treatment	Milk conc. ^b	Initial solubility index	
			Milk conc. ^b	Powder
		(% T.S.)		
106	160° F.	23.3	98.6	97.9
107	30 min.	40.1	99.5	99.3
98	170° F.	22.8	98.9	98.7
99		38.9	99.4	98.9
100		23.4	98.3	97.5
101	10 min.	42.0	99.1	99.2
104		22.7	99.1	98.5
105		39.4	99.3	99.4
94	180° F.	20.6	98.4	99.2
95		41.6	99.3	99.1
96		21.4	99.1	98.1
97		39.9	99.2	99.1
102		39.0	99.1	99.4
103	10 min.	26.5	99.2	99.3
108		22.6	98.9	97.6
109		38.6	99.3	97.9

^a The powders are paired. The even-numbered powders and the following odd-numbered powders are made from the same milk.

^b Homogenized.

trated milks and the corresponding powders made from them. The solubility indices obtained with the concentrated fluid milk samples no. 94 to 109, inclusive, range from 98.3 to 99.5. Generally, the less concentrated samples gave lower values than did the more concentrated samples. The effect of concentration of the milk on the solubility of the powder was even greater than when the test was applied only to the concentrated milk. More efficient homogenization of the more viscous, heavier concentrate may have resulted in the differences of the solubility indices of the milks and the greater differences observed in the powders made from the 20 and 40 per cent total solids concentrate.

Color and solubility. The powders made in this experiment had moisture contents which ranged from 1.5 to 3.1 per cent. None of the powders was discolored noticeably after storage for 180 days at 100° F. Krienke and Tracy (9) showed that brown discoloration of powder takes place at all storage temperatures, the discoloration increasing with increased moisture in the powder. Discoloration was accompanied by decreasing solubility. Hollender and Tracy (6) stated that it was evident that there was a critical point in the neighborhood of 5 per cent moisture content which represented the upper limit of moisture for samples stored at room temperature without deterioration in flavor and color. However, they found discoloration of milk powders would occur at 68° F. or higher, regardless of moisture content. They stored samples of milk powder with moisture contents varying from 2.32 to 5.39 per cent at 98.6° F. All of their samples discolored within 67 days. The lower average moisture content of the present samples may have prevented noticeable discoloration.

Density and particle size. The apparent density of the powdered milk is important for it affects packing volume and ease of handling as well as ease of reconstitution. The effects on packing volume and ease of handling or packaging are well known in the commercial field.

Webb and Hufnagel (18) found that increasing preconcentration of the milk resulted in powders with increased densities. In agreement with their findings, it was found in this work that with a given preheat treatment there was a direct correlation between density and preconcentration of the milk (table 4). This direct correlation of density with degree of precondensing was noted in every instance except for powder no. 94. In the production of this powder, trouble was experienced with continuous clogging of the air outlet of the spray nozzle. It is believed that this prevented normal atomization of the milk, resulting in a heavier, coarser powder.

Increasing the degree of preconcentration from 20 to 40 per cent total solids resulted in whole milk powder not only of greater density but with a higher percentage of the larger-size powder particles (table 5). Wilster *et al.* (19) stated that one of the factors favorable to reconstitutability of dry milk was small particle size. The powders with the highest solubility indices, as reported in this paper, had the highest percentage of the large size particles. However, it must be noted that in nearly all of the powders examined, 95 per cent of the particles were less than 10 μ in diameter (table 5). All of the

TABLE 4
Whole milk powder densities

Powder no.	Preheat treat.	Preconc.	Powder		Densities at 25° C.	Av. density	
			% fat ^a	% T. S.		sample	group
		(% T. S.)					
70		21.9			1.161, 1.162, 1.162	1.162	
72		18.0			1.180, 1.178, 1.181	1.180	
76	160° F.	19.9	26.3	97.7	1.176, 1.175, 1.170	1.174	
106		23.3	28.4	97.5	1.152, 1.153	1.153	1.167
	for						
71		38.9			1.214, spilled	1.214	
73	30 min.	38.6			1.216, 1.213, 1.211, 1.212, 1.215	1.213	
77		39.3	26.3	97.8	1.206, 1.209, 1.208	1.208	
107		40.1	28.4	97.6	1.189, 1.188	1.188	1.206
88		19.6	26.5	97.9	1.162, 1.165, 1.163	1.163	
92		20.5	26.3	97.9	1.163, 1.160, 1.162	1.162	
100	170° F.	23.4	29.4	97.6	1.135, 1.136	1.136	
110		23.9	28.3	97.6	1.155, 1.158, 1.157	1.157	1.154
	for						
89		42.2	26.6	98.2	1.198, 1.197, 1.201	1.199	
93	10 min.	41.3	26.5	98.5	1.198, 1.199	1.199	
101		42.0	29.5	97.9	1.185, 1.188, 1.186	1.186	
111		37.4	28.3	97.5	1.193, 1.193, 1.192	1.193	1.194
74		20.8			1.178, 1.178, 1.177	1.178	
78		22.0	26.4	97.6	1.169, 1.170, 1.170	1.170	
80		21.0	26.1	97.7	1.164, 1.165, 1.165	1.165	
82	170° F.	19.3	26.1	97.8	1.159, 1.160	1.160	
84		20.3	26.1	97.9	1.158, 1.158	1.158	
104		22.7	27.9	97.5	1.157, 1.155	1.156	1.165
	for						
75		44.4			1.195, 1.192, 1.193	1.193	
79	30 min.	40.7	26.5	97.9	1.204, spilled	1.204	
81		40.3	26.2	98.2	1.206, 1.209	1.208	
83		40.3	26.2	98.1	1.199, 1.195	1.197	
85		40.3	26.1	98.0	1.202, 1.201, 1.202	1.202	
105		39.4	27.9	97.7	1.192, 1.194	1.193	1.198
86		22.0	28.1	97.8	1.164, 1.159	1.162	
90		22.1	26.7	97.4	1.154, 1.157	1.156	
90 ^b		22.1	26.7	97.4	1.152, 1.155	1.154	
94 ^c		20.6	28.4	98.1	1.196, 1.194, 1.197, 1.193, 1.195, 1.196	1.195	
98		22.8	28.3	97.9	1.139, 1.140, 1.138, 1.138, 1.141, 1.141	1.140	
103	180° F.	26.5	28.3	97.2	1.162, 1.164	1.163	
108		22.5	29.0	97.6	1.151, 1.151	1.151	1.154
	for						
87		41.6	28.2	98.4	1.189, 1.190	1.190	
91	10 min.	41.9	27.8	98.2	1.196, 1.197	1.197	
95		41.6	27.5	98.0	1.182, 1.182, 1.179, 1.182	1.181	
99		38.9	28.3	98.0	1.187, 1.189	1.188	
102		39.0	28.4	97.4	1.189, 1.189	1.189	
109		38.6	29.0	97.8	1.189, 1.188	1.188	1.189

^a Calculated per cent fat based on per cent fat in the fresh milk.

^b Adsorbed and interstitial air removed by centrifugation.

^c During the production of this powder, the air outlet of the atomizing nozzle was apparently out of adjustment and became clogged continuously, preventing normal atomization. This result, therefore, is not included in the average.

powder produced for this experiment may be classified as of small particle size, for Hunziker (8) indicates the size of milk powder particles produced commercially by the spray process ranges from 10 to 100 μ , with 80 per cent of the particles being in the relatively coarse group.

In the solubility tests, it was noted that the powders from the 20 per cent concentrate always were highly charged. Even the slightest handling of this lighter powder markedly increased the static charge and made the handling of this powder difficult. Consequently, the dense powder may play a favorable role in the reconstitutability of whole milk powder, because its lesser tendency

TABLE 5
Size distribution of powder particles

Powder no.	Preheat treat.	Preconc.	% of particles—Diameters in microns						
			0-5	5-10	10-15	15-20	20-25	25-30	30-45
		(% T.S.)							
72		18.0	83.0	15.2	1.7	0.1			
70	160° F.	21.9	79.1	16.3	3.6	0.7	0.24		
73	30 min.	38.7	68.4	26.2	4.1	0.8	0.53		
77		39.3	61.4	29.7	6.0	1.8	0.50	0.50	
88		19.6	88.0	11.5	0.5				
110	170° F.	23.9	82.6	16.5	0.5	0.1			
111	10 min.	37.4	61.9	31.3	5.5	1.2	0.10		
101		42.0	67.1	25.4	4.5	2.1	0.33	0.47	0.21
74		20.8	81.7	16.5	1.5	0.4	0.03		
80	170° F.	21.0	80.6	18.1	1.1	0.2	0.09		
85	30 min.	44.3	70.4	23.4	4.6	1.1	0.39	0.16	0.02
75		44.4	65.9	27.2	5.1	1.0	0.47	0.16	0.10
94		20.6	74.9	21.6	3.1	0.3	0.04		
98	180° F.	22.8	83.4	14.9	1.5	0.2	0.08		
109	10 min.	38.6	73.9	22.5	2.9	0.5	0.13		
95		41.6	72.2	21.5	3.7	0.9	0.75	0.25	0.57

to build up a high electrostatic charge appears to reduce the wettability of the powder, and the dense powder naturally has less occluded air.

SUMMARY AND CONCLUSIONS

(1) Precondensing milk to a level of 40 per cent total solids resulted in a spray-dried powder of higher solubility than when the milk was precondensed to a level of 20 per cent total solids.

(2) The powders made from the milk of all preheat treatment levels and concentrated to the 40 per cent level reconstituted quickly and without a visible film of specks on the glassware. For all practical purposes, they were 100 per cent soluble at the end of 6 months of storage at 45° F.

(3) The solubility of the powders made from milk preheated at the lower temperatures and precondensed to 40 per cent total solids did not decrease appreciably when stored for 6 months at 100° F. A preheat treatment at 180° F. for 10 minutes appears to induce heat denaturation of the protein, which is con-

tinued when the powder is stored at 100° F., resulting in continued loss of solubility during storage.

(4) A method for quickly and accurately determining the density of whole milk powder is presented.

(5) The density of milk powder increases with increasing preconcentration of the milk. The powders made from milk concentrated to 40 per cent total solids were easier to reconstitute, did not take up as much space, and did not as readily develop a high electrostatic charge as did the powders made from the 20 per cent total solids concentrate.

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EFFECT OF THYROXINE ON OXYGEN CONSUMPTION OF BOVINE SPERMATOZOA AND SEMEN¹

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Previous studies have shown that the rate of oxygen consumption of spermatozoa may be related to fertility (3, 9). Other studies have shown that thyroxine increases the oxygen consumption of some tissues *in vitro* (1, 2). Since certain oxidative mechanisms may play a role in spermatozoan physiology similar to that in other respiring cells (4, 5), the purpose of this experiment was to determine the effect of thyroxine on oxygen consumption and, ultimately, to study the effect of thyroxine on semen fertility. The results obtained with a measured amount of thyroxine on the oxygen consumption of bovine spermatozoa and semen are reported in this paper.

METHODS

Bovine semen was collected by use of the artificial vagina under conditions as aseptic as possible. Experiments with washed and unwashed spermatozoa were carried out. In experiments with washed spermatozoa, the semen was cooled slowly to 4.5° C. immediately after collection and diluted with a phosphate buffer.² The diluted semen was centrifuged and the diluted seminal fluid removed. Phosphate buffer then was added in amounts to make up the original diluted volume. Experiments on unwashed semen were made on samples collected as described above and diluted with a diluent composed of one part fresh egg yolk to ten parts phosphate buffer. The rate of semen dilution varied for different samples. Since preliminary experiments indicated that thyroxine exerts its effect on respiration rate only after it has been in contact with the spermatozoa for several hours, the determinations in these experiments were made 10 to 30 hours after addition of thyroxine to the semen or spermatozoa. Only a limited number of the centrifuged and washed semen samples maintained respiratory activity at a level high enough for a reliable determination after 10 to 30 hours of storage. Preliminary work (7) indicated that from 0.3 to 1.0 γ of DL-thyroxine in 10 ml. of diluted semen brought about an average increase in oxygen consumption. Therefore, 0.7 γ of DL-thyroxine per 10 ml. diluted semen or washed spermatozoa was used in all of the determinations.

The DL-thyroxine was prepared by isolation from iodocasein which was thyroidally active.³ A weighed amount of this thyroxine was added to distilled water, completely dissolved by adding 0.1 N NaOH drop by drop and this solu-

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² Made up of 20 g. $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ plus 2 g. KH_2PO_4 in 1,000 ml. boiled distilled water.

³ Supplied by Dr. E. P. Reineke of Michigan State College, East Lansing

tion made up to a volume giving 100 γ thyroxine per ml. For use in treating semen, this thyroxine solution was diluted further with a phosphate buffer so that 1 ml. of solution contained 5 γ . A portion of each semen sample was treated with thyroxine and another portion used as a control. The pH was adjusted so that it was the same in treated and control portions.

Oxygen consumption was determined with a Barcroft-Warburg respirometer at 37° C. The manometers were shaken at a rate of 110 oscillations per minute. Care was taken to mix the semen thoroughly before dividing it into portions. To determine the variability of results due to sampling and operation of the respirometer, the oxygen consumption of two untreated portions of a semen sample was determined. The standard deviation of the difference between two like samples for ten determinations was found to be 1.24 mm.³ oxygen. The concentration of spermatozoa in the original semen was determined with a hemocytometer.

To determine the influence of bacterial contamination on results, oxygen consumption determinations were made on thyroxine-treated and untreated portions of semen in which the spermatozoa had been killed by adding a measured quantity of water to the raw semen before dilution or by warming and cooling of the semen. Also, oxygen consumption determinations were made on treated and control semen to which 800 units of penicillin per ml. had been added. Dilution was one part semen to four parts buffer.

EXPERIMENTAL

The effect of thyroxine in a concentration of 7 γ per cent on the oxygen consumption of bovine spermatozoa and semen is shown for individual semen samples in table 1. On a basis of the oxygen consumed per 100 million spermatozoa per hour, the results show that the average was 8.48 mm.³ for control semen and 9.20 mm.³ for thyroxine-treated semen. These values were obtained with semen stored from 10 to 30 hours at 4.5° C. and with varying concentration of spermatozoa. On the same basis, control semen consumed an average of 8.12 mm.³ oxygen when the concentration of spermatozoa in the original semen was below 800,000 per mm.³, whereas these same semen samples when treated with thyroxine consumed 8.00 mm.³. Apparently, no average change in the amount of oxygen consumed was brought about with the addition of thyroxine. Control semen with a spermatozoan concentration of more than 800,000 per mm.³ and less than 1,400,000 per mm.³ in the original semen consumed 8.66 mm.³ oxygen, whereas these same semen samples treated with thyroxine consumed 9.64 mm.³ oxygen, an average increase of 11.2 per cent.

The results obtained were correlated with spermatozoan concentration of the original semen. In general, as the spermatozoan concentration of the original semen increased from 800,000 to 1,400,000 per mm.³, there was a progressively greater increase in oxygen consumption resulting from the presence of DL-thyroxine. With semen having a spermatozoan concentration of less than 800,000 per mm.³, there was little or no change in the oxygen consumption due to the presence of thyroxine. The correlation coefficient representing the degree of this relationship was found to be +0.60. This is a statistically highly sig-

nificant correlation for the number of determinations involved. This relationship apparently was not due to spermatozoa number *per se*, because the actual number of spermatozoa present in each determination was not related to increasing oxygen consumption resulting from thyroxine treatment. The correlation coefficient representing the degree of the latter relationship was +0.19. Therefore, it is believed that differences in changes of oxygen consumption due to thyroxine are due to variations of some character of semen that is associated

TABLE 1
Effect of 7 γ DL-thyroxine per 100 ml. of diluted semen on oxygen consumption

Sperm concentration in original semen	No. of sperm per 2 ml. diluted sample	Oxygen consumption		% of control
		Control	Treated	
(Thousands/ mm. ³)	(Millions)	(mm. ³)	(mm. ³)	
<i>Washed, diluted semen</i>				
700	280	26.56	23.54	88.6
800	266	12.16	14.06	115.6
800	266	24.60	27.68	112.5
900	450	33.47	35.76	106.8
1,000	600	32.06	36.09	112.6
1,160	1,160	29.41	31.22	106.1
<i>Unwashed, diluted semen</i>				
300	120	11.44	11.08	96.9
350	170	14.36	13.84	96.4
450	360	23.34	26.06	111.7
550	220	23.99	23.57	98.3
688	688	36.74	33.94	92.4
700	350	24.28	22.73	93.6
725	482	37.61	42.39	112.7
800	400	75.81	72.63	95.8
820	410	28.58	28.39	99.3
900	360	16.68	21.37	128.0
980	290	21.36	22.86	107.0
1,000	1,000	52.84	59.79	113.2
1,000	100	20.27	22.00	108.5
1,100	220	27.08	32.48	119.9
1,100	440	46.46	48.30	103.9
1,000	440	27.69	34.47	124.5
1,130	900	65.68	75.62	115.1
1,200	480	45.61	50.45	110.6
1,220	490	29.53	34.27	116.1
1,220	1,220	43.25	51.11	118.2
1,375	680	111.60	136.40	122.2

with spermatozoan concentration of the original sample and not to sperm numbers *per se*.

Although not enough evidence has been obtained on semen from individual bulls to draw definite conclusions, it appears that semen from some bulls usually increases in its ability to consume oxygen when thyroxine is present, and semen from these bulls usually has a high spermatozoan concentration. Other bulls seem to produce semen that is depressed or not influenced in its ability to consume oxygen when thyroxine is added and usually is low in spermatozoan concentration. In cells such as bovine spermatozoa that vary considerably in their

physiology, as is indicated by their varying ability to effect fertilization, it is not surprising that the reaction to experimental conditions varies. Varying effects on the oxygen consumption of different tissues due to the influence of thyroxine have been reported by other workers (2, 8).

Bacterial contamination cannot be avoided entirely in semen collection, and its influence on total oxygen consumption may affect results. Therefore, the oxygen consumption of semen samples with the spermatozoa killed was determined for 12 samples. One portion of the sample containing dead spermatozoa was treated with thyroxine and another untreated portion served as a control. The average oxygen consumption of these 12 samples with dead spermatozoa was 3.26 mm.³ per hour, with a standard deviation from the mean of 1.97 mm.³. Actual variation was from an amount of oxygen which could not be detected to 6 mm.³ per hour. For these same semen samples with thyroxine added, the average oxygen consumption was 3.68 mm.³ per hour. With semen to which penicillin had been added immediately after collection and dilution, there appeared to be a similar response to thyroxine, except that the percentage increase in oxygen consumption with thyroxine was less than that to which no penicillin had been added. Increases of from 2 to 12 per cent in the oxygen uptake were obtained with 8 semen samples. The decreased response may be due to the interference by penicillin with oxidative mechanisms of the spermatozoa as well as that of bacteria.

The results obtained in these experiments indicate that total oxygen consumption of spermatozoa is influenced by contaminating substances. However, since washed spermatozoa, as well as unwashed and penicillin-treated semen, showed a similar pattern of response to added thyroxine, it is assumed that spermatozoan metabolism was affected by the treatment.

Wide variability between the oxygen consumption of individual semen samples is influenced by individuality of the semen sample, length of time the semen was stored, the rate of dilution (6) and probably by unavoidable differences in handling individual semen samples.

SUMMARY

Bovine spermatozoa and semen, diluted with a phosphate buffer, were treated with 7 γ per cent DL-thyroxine.

Oxygen consumption generally was increased with the addition of thyroxine in semen samples with an original spermatozoan concentration of from 800,000 to 1,400,000 per mm.³

Semen samples with a spermatozoan concentration of less than 800,000 per mm.³ in the original semen were not influenced on the average by the presence of thyroxine in the concentration used.

The ability of semen to respond with increased respiration rate to added thyroxine appears to be related to some factor that is associated with original spermatozoan concentration.

The influence of bacterial and other contamination on results has been discussed. Its exact influence remains unevaluated.

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RETENTION OF ASCORBIC ACID, CHANGES IN OXIDATION-REDUCTION POTENTIAL, AND THE PREVENTION OF AN OXIDIZED FLAVOR DURING FREEZING PRESERVATION OF MILK

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Outstanding among beverage milk problems is the prevention of the flavor described as oxidized, tallowy or cappy, that tends to develop in milk that is produced under sanitary conditions and has a low bacterial content.

Preservation of milk by freezing is a means of studying the oxidized flavor problem which has been little utilized. In the frozen state, milk is more stable than in the unfrozen state, and its changing characteristics can be studied over a longer period. Babcock *et al.* (1), using samples packaged in paper at a large dairy, found the milk of acceptable beverage quality after storage at -32.8°C . for 115 days. At higher temperatures frozen milk is not as stable and, in the author's experience, always eventually becomes oxidized in flavor. The onset of this defect is a limiting factor in the preservation of milk by freezing and, therefore, is of economic importance.

There are at least four forms of ascorbic acid—two that are levorotatory and are called ascorbic acid and dehydroascorbic acid and two that are dextrorotatory. The latter two are not biologically active. Of the former, each of which is equally biologically active, ascorbic acid is the only form of vitamin C present in milk in the mammary gland (6). The oxidized flavor ordinarily is detected in milk containing dehydroascorbic acid, the first oxidation product of ascorbic acid, as well as ascorbic acid. Krukovsky and Guthrie (7) concluded that ascorbic acid oxidation is an essential link in the chain of the reactions resulting in the development of the tallowy (oxidized) flavor in milk, and that apparently the oxidation of the lipid fraction of milk is coupled to that of ascorbic acid when a certain equilibrium between ascorbic acid and dehydroascorbic acid has been established.

Greenbank (4) believes that the development of an oxidized flavor in milk is related to a change in the oxidation-reduction potential, and that variations in milks can be explained on the basis of differences in their poisoning action.

Dahle and Palmer (3) have expressed the situation as follows: "A condition in the milk which is favorable to the production of the oxidized flavor is apparently favorable to the destruction of vitamin C." They found that the ascorbic acid content gradually decreased during the holding period in all cases, regardless of the development of off-flavor. The reduction in vitamin C was nearly as great in the normal milk as in the milk that developed the off-flavor.

EXPERIMENTAL METHODS

The titration of the milk for reduced ascorbic acid was carried out according

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to the procedure published by Sharp (9). The solution of sodium 2,6-dichlorophenolindophenol was prepared and standardized as described by Stewart and Sharp (11).

In obtaining the milk, no attempt was made to select milk from individual cows, based on their tendency to produce milk that readily developed an oxidized flavor. Morning milk was utilized and was processed in the course of the forenoon. Copper contamination was avoided by employing hand milking and by having the milk delivered in the can into which it was poured from the milk pails.

To delay and prevent the onset of an oxidized flavor, Sharp *et al.* (10) deaerated milk. A convenient laboratory apparatus for deaeration of milk is the one devised by Mottern and Von Loesecke for deaeration of citrus juices (8). This was utilized in the present work.

In deaerating the milk, 12-l. balloon flasks were used and the vacuum was 29 inches. The warm deaerated milk was poured directly into clean well-tinned cans of about 150 ml. capacity, the cans sealed and their contents frozen. Three cold-air storage spaces were utilized: one had a temperature of -10° C., another, -16° C., and the third, -27° C. The temperature of the air in these spaces varied several degrees Fahrenheit. The ascorbic acid was dissolved in a small volume of distilled water and added to the milk.

Unhomogenized milk was found less suitable for these experiments than homogenized milk, since it is less resistant to the onset of the oxidized flavor (13) and, on thawing, shows inferior distribution of insoluble solids. Homogenization was effected at 2,500 lb. pressure per square inch immediately after heating the milk at 62° C. for 30 minutes. The frozen milks were thawed by immersing the cans in warm water.

Oxidation-reduction (E_h) measurements were made with a battery-operated pH meter, using a calomel half-cell. One end of an agar-KCl glass bridge rested in a saturated potassium chloride solution and the other in the sample of milk in a glass beaker. Also partially submerged in the potassium chloride solution was the calomel half-cell and, in the milk was a gold foil electrode fused to a gold wire; these were connected to the jacks of the meter. Each day the electrodes were used, they first were cleaned in the flame of an alcohol lamp, this flame being more suitable than the less pure flame of a Bunsen burner. Each E_h value in the following data is the average of three readings obtained with three electrodes. With but few exceptions, results with the three electrodes were in excellent agreement.

The E_h values of a set of thawed milk samples were obtained either late in the forenoon and again in the afternoon or in the course of the same afternoon but an hour or two apart. In case of differences between the two readings on the same sample, an average value usually was regarded as most representative. The older the samples, the greater this difference was likely to be.

The hand-drawn, uncooled morning milk was processed as follows: *C* (the control)—At the end of the holding period of 30 minutes at 62° C., the milk was homogenized at 2,500 lb. pressure per square inch, cooled, canned and

frozen. *CD*—Similar to *C* except that it was deaerated as already described above its boiling point (50° C.) just before it was canned. *P 20*—Same as the control, except that a solution of ascorbic acid was added to the cooled milk at the rate of 20 mg. per l. of milk. *P 20 D*—Same as *P 20* except that it was deaerated above its boiling point after the addition of the vitamin. *P 40*—Same as *P 20* except that 40 mg. of ascorbic acid per l. of milk was added instead of 20. *P 40 D*—Same as *P 40* except that the warm milk was deaerated after the addition of the vitamin. *20 P*—Same as *P 20* except that the ascorbic acid was added to the raw milk. *20 PD*—Same as *20 P* except for deaeration just before canning. *40 P*—Same as *P 40* except that the vitamin was added to the raw milk. *40 PD*—Same as *40 P* except that the vitamin fortified milk was deaerated before it was canned.

EXPERIMENTAL RESULTS

Figure 1 presents the results of an experiment designed to show the retention at -27° C. of ascorbic acid in milk prepared under various conditions and the

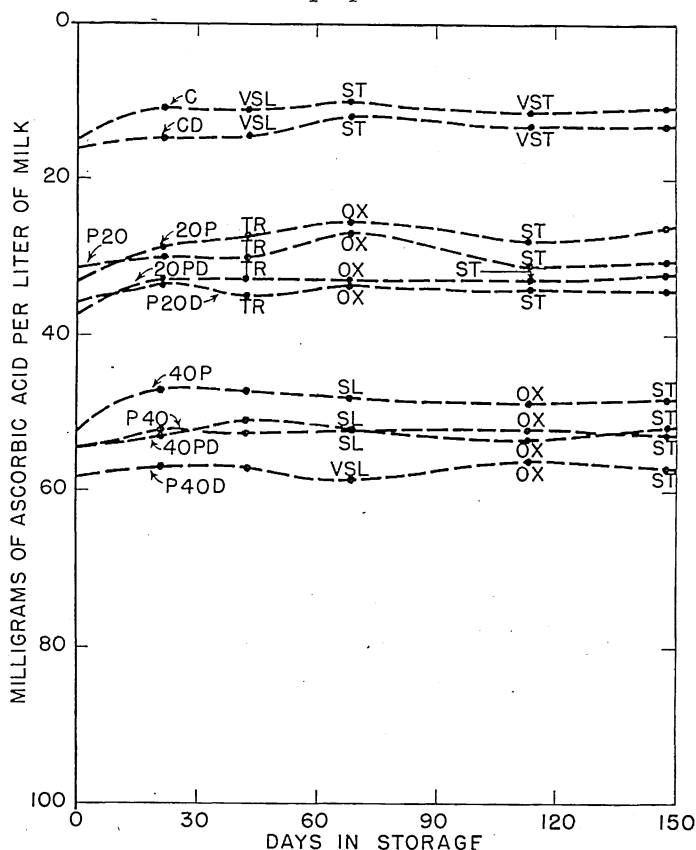


FIG. 1. Retention of ascorbic acid in milk stored at -27° C. and the effect of deaeration and of fortifying the milk with ascorbic acid upon the rate of development of an oxidized flavor. Oxidized flavor intensity is indicated on the curves as follows: tr = trace, v sl = very slight, sl = slight, ox = oxidized, st = strongly oxidized, and v st = very strongly oxidized.

rate of development of an oxidized flavor. To hasten the rate of freezing, these cans of warm milk were immersed in alcohol at -16°C . to which dry ice was added to obtain agitation and to prevent a rise in temperature. Samples were examined after they had been in storage 21, 42, 68, 112 and 147 days. As soon as each fresh milk had been prepared, a small portion was placed in a room maintained at 2 to 4°C . After 9 days, only staleness was detectable in these fluid samples. They were not oxidized.

The ascorbic acid content of the fresh samples was determined 3 to 4 hours after they were placed in the cold room. There was substantially more ascorbic acid in the deaerated than in the undeaerated fresh milks, and this difference was essentially the same in all the thawed samples that were examined during the storage period. Little significance is attached to the apparent advantage of adding ascorbic acid after pasteurization rather than before. Additional evidence on this point is needed.

A straight line drawn from the beginning of each curve to its end would be

TABLE 1
*Reduced ascorbic acid content and E_h of the samples of experiment 2
after 4 days at 2 to 4°C .*

Sample no.	Reduced ascorbic acid	Oxidation-reduction potential
	(mg./l.)	(millivolts)
C	0.0	284
CD	0.0	281
P 20	0.0	289
P 20 D	2.4	278
P 40	4.2	280
P 40 D	13.8	256
P 60	16.2	252
P 60 D	31.8	237

nearly horizontal, especially those lines representing portions of the milk that were fortified with ascorbic acid after pasteurization. Although the concentration of ascorbic acid decreased only slightly, these samples all developed a strong oxidized flavor but at different rates. On a percentage basis, ascorbic acid retention was least in the control and greatest in the deaerated milk that was fortified after pasteurization.

The body of all the thawed samples was good. There was no apparent flakiness or fat separation.

Figures 2, 3, and 4 illustrate the relationship in thawed milk between retention of ascorbic acid, the oxidation-reduction potential and the onset of the oxidized flavor. The morning milk was hand-drawn and, except as indicated, was processed and labeled as in the preceding experiment. All canned samples were in a hardening room before noon for freezing and storage at -16°C . Four days after samples of the freshly prepared milks were placed in the 2 to 4°C . room there was a trace of an oxidized flavor in the control, and the deaerated samples were not as flat in flavor as the non-deaerated ones. Their ascorbic acid content and E_h values are given in table 1.

The reduction in E_h in the freshly prepared samples, as additional units of ascorbic acid were added, is shown in figure 2. The average of several measurements on different milks, in which increments of 25 mg. of ascorbic acid per l. of milk instead of 20 were added, is as follows: first, 25 mg./l., 38 millivolts; second, 25 mg./l., 20 millivolts; third, 25 mg./l., 11 millivolts; and fourth, 25 mg./l., 7 millivolts.

After 23 days in storage, a set of samples was thawed by placing the cans in warm water. Each can was shaken, opened and a portion poured into a small

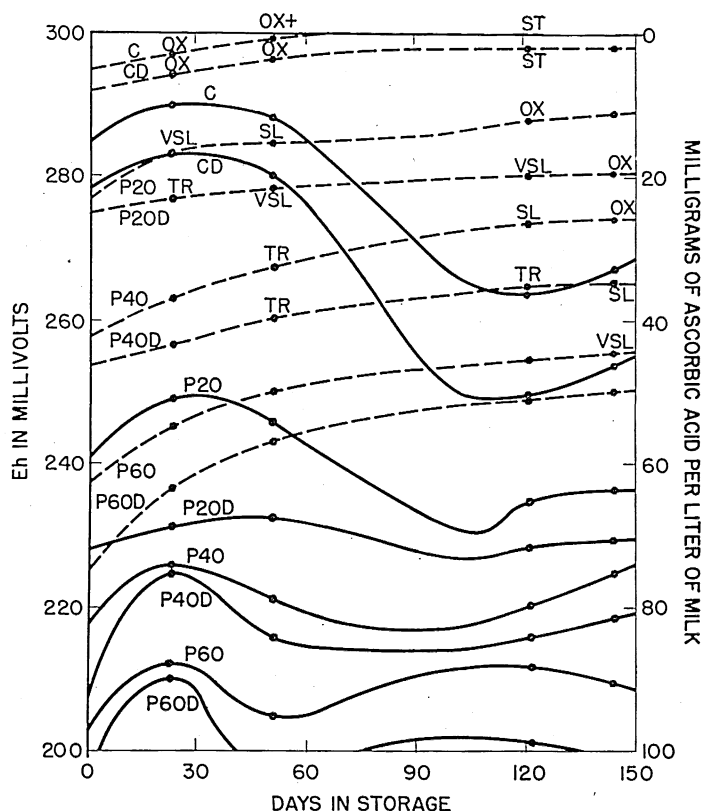


FIG. 2. Retention of ascorbic acid, changes in E_h , and the development of an oxidized flavor in milk stored at -16°C . Broken lines represent mg./l. of ascorbic acid, solid lines the E_h of the samples. (Flavor designations as in fig. 1.)

beaker, after which the opened cans were covered and stored at 2 to 4°C . The beaker samples were warmed to 30°C . and their ascorbic acid content and oxidation-reduction (E_h) values determined. The titrations showed losses in the reducing agent (ascorbic acid), and the E_h measurements disclosed increases in oxidation-reduction potential. Examinations of samples thawed at later dates indicated a continuing decrease in ascorbic acid content but a decrease in E_h followed by an increase. At the same time, an oxidized flavor became detectable and increased in intensity in most of the milks, the rate of increase being greatest

in the control. When a sample which had been fortified by the addition of 60 mg. of ascorbic acid per l. of milk and then deaerated before it was frozen was examined after 160 days in storage, no trace of an oxidized flavor was found. After 51 days in storage, the older these samples were when they were thawed, the greater was the separation of insoluble solids.

Two and 3 days and again 4 to 7 days after each set of thawed milks had been placed at 2 to 4° C., beaker samples were obtained, warmed to 30° C. and the concentration of ascorbic acid and the oxidation-reduction potentials re-determined. These data are plotted in figures 3 and 4. Figure 3 also shows additional

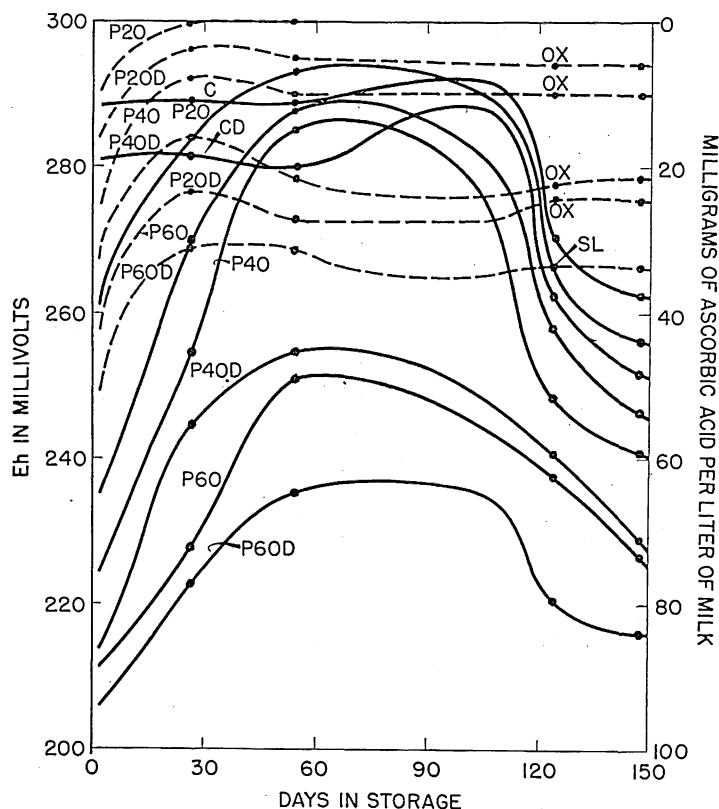


FIG. 3. Ascorbic acid content, E_h , and intensity of the oxidized flavor of the thawed samples of figure 2 after 2 to 3 days at 2 to 4° C. (Flavor designations as in fig. 1.)

data on the flavor of the samples. In general, as the amount of ascorbic acid in these thawed refrigerated samples decreased, the oxidation-reduction potential increased, and the intensity of the oxidized flavor increased. A decrease in the intensity of the oxidized flavor never was noted. Having once developed, it always became more pronounced.

Figure 5 shows the effect of the storage temperature upon the retention of ascorbic acid, the oxidation-reduction potential and the onset of an oxidized flavor in samples of the same milk containing increasing amounts of added ascorbic acid. Methods used in earlier experiments were followed in preparing

and examining the samples. As soon as the various milks were ready, several cans of each, representing controls and the variables, were placed in compartments maintained at -10 , -16 and -27°C . When 9 days old, a set of refrigerated fluid samples included no milk that tasted oxidized or contained titratable ascorbic acid. The E_h values ranged from 297 to 305 millivolts. Samples stored at -10°C for 44 days and then thawed were flaky; those held at -16°C for 98 days also were flaky, but all samples that were thawed after storage at -27°C were homogeneous. As in other experiments with but few exceptions, the more

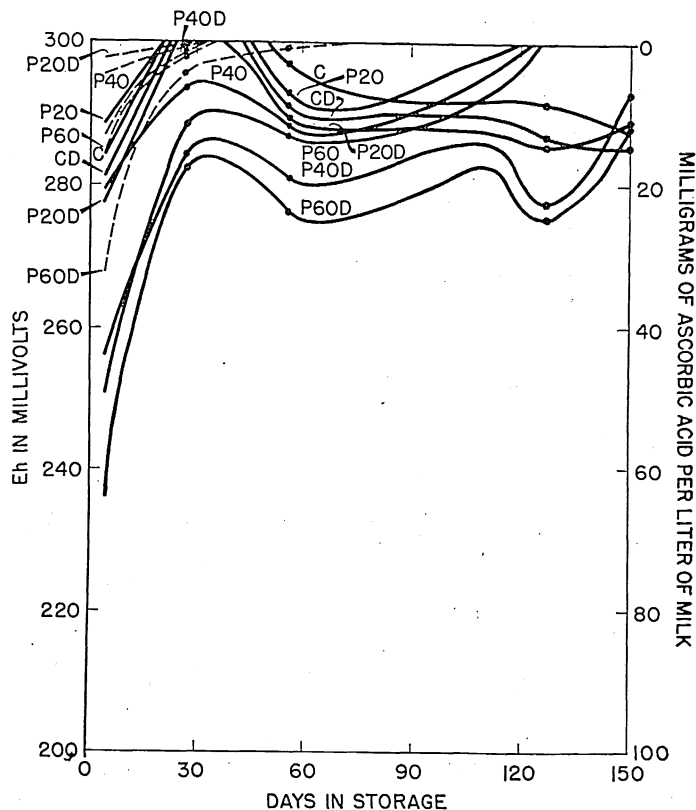


FIG. 4. Ascorbic acid content and E_h of the thawed samples of fig. 2 after 4 to 7 days at 2 to 4°C .

the milk was fortified with ascorbic acid and the lower its storage temperature, the greater was the resistance of the milk to the onset of an oxidized flavor.

The evidence in figure 5 indicates that oxidation of ascorbic acid and the onset of the oxidized flavor in milk may be accompanied by a reduction in the electromotive force or chemical energy of the system. After 98 days in storage, during which there was a loss in ascorbic acid and the development of an oxidized flavor, most of the samples had a lower oxidation-reduction potential than they did when they were frozen.

DISCUSSION

Many who have referred to the off-flavor known as "oxidized" or "tallowy"

have taken it for granted that oxidation of the fat was the cause. The view that the true fat is involved is unsound. Milk fat is too stable to be affected by this mild reaction (5). There is considerable evidence that a phospholipid, a relatively unstable fat-like substance containing phosphorus and associated with the fat, is the constituent of milk that is affected.

Although oxidation of a phospholipid, probably lecithin, now quite generally is regarded as the cause of the defect, oxidation-reduction potential measurements have been used by only a relatively few investigators in studying the problem. This paper attempts to show oxidation-reduction changes during the onset of the off-flavor in frozen milk by examining its thawed product, and at the same time recording the decrease in ascorbic acid.

In the experiment on which figure 1 is based, it was demonstrated that a

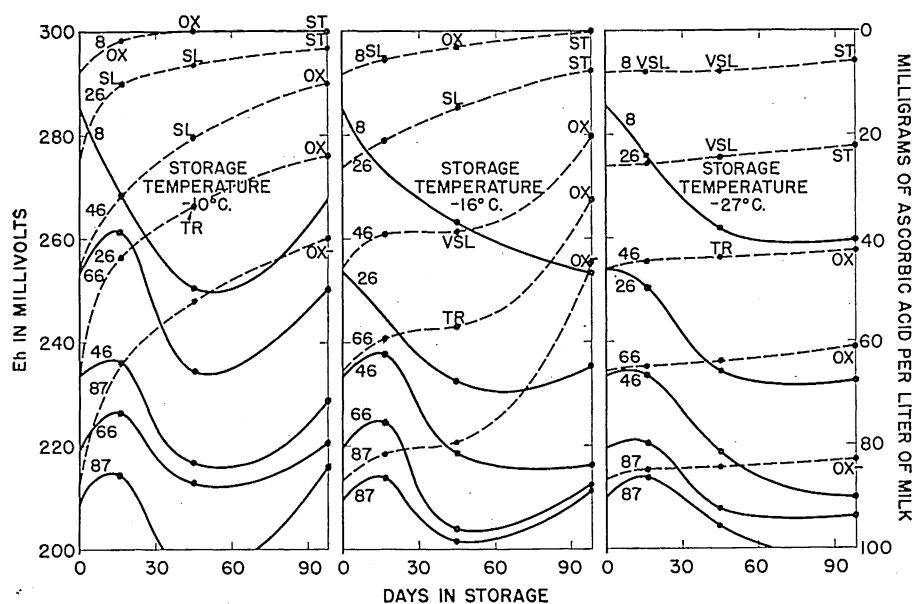


FIG. 5. Relationship between the retention of ascorbic acid, the oxidation-reduction potential and the onset of an oxidized flavor in milk stored at different temperatures. The corresponding ascorbic acid and E_h curves are indicated by numbers which also represent the mg. per l., of ascorbic acid in the fresh samples. Broken lines represent ascorbic acid, solid lines oxidation-reduction potential (E_h) of the samples. (Flavor designations as in fig. 1.)

strong oxidized flavor may develop in milk with but slight decrease in ascorbic acid content; also, the rate of development is much slower in milk that has been fortified with the acid. Deaeration aided in the preservation of the acid but only slightly retarded the onset of the off-flavor.

The storage temperature in the second experiment was not as cold as in the first; figure 2 shows that the retention of ascorbic acid was not as effective. Here again, fortification with ascorbic acid protected the fresh flavor of the milk. Although the E_h values, relative to their initial relationships, continued to follow approximately the same pattern, they did not continue to increase but rather decreased and then began to increase again.

Insofar as these data are concerned, it appears that during storage frozen milk may become more oxidized in flavor while its oxidation-reduction potential is decreasing. Whether or not the E_h values of the thawed samples at 30° C. accurately reflect the E_h values during frozen storage is not known. If they do, then opposing reactions were going on during a large part of the storage period. One reaction is a slow oxidation of ascorbic acid to dehydroascorbic acid and the other (or others) is reducing in character and of greater effect upon the oxidation-reduction potential of the system. If E_h values after thawing do not reflect the E_h values during storage, then constituents redissolved and soon approached a new equilibrium that substantially altered the oxidation-reduction potential. Dahle, *et al.* (2), working with frozen cream, obtained results that resemble these on frozen milk.

In the present study all E_h measurements were made in the same manner, and no reading was acceptable if there was drifting and the electrodes were not in agreement. The data, therefore, should be relative.

Swanson and Sommer (12) conclude from their studies on oxidation-reduction potentials in relation to the development of oxidized flavor in fluid milk that the E_h value of the medium does not seem to inhibit or accelerate the development of the off-flavor.

CONCLUSIONS

From the data of this paper, it may be concluded that a low E_h , obtained by adding ascorbic acid, greatly defers but does not prevent the development of an oxidized flavor in frozen milk. However, a low E_h does not increase the retention of vitamin C in the form of ascorbic acid.

In determining the flavor of the milks, the author had the benefit of the experienced judgment of C. J. Babcock.

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LIPID DETERIORATION IN DAIRY PRODUCTS. THE STABILITY OF MILK FAT AND FAT-SOLUBLE VITAMINS AS DETERMINED BY THE RE-EMULSIFICATION TEST

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The milk excreted by the mammary gland under proper sanitary and feeding conditions has a pleasant and sweet taste. Quite often, however, milk and its products develop flavors which render them distasteful to the human palate and are the cause of their rejection. There are rancid and oxidized flavors. While it is possible to reduce to a minimum, retard or prevent the lipolysis by a quick and prompt cooling of raw milk, storage at constant temperatures (2, 8) and subsequent pasteurization treatments (9), uniform control of the development of oxidized flavors is not possible.

The development of oxidized flavors in fresh milk could be practically retarded or prevented by the deaeration of milk (11), or depletion of milk of its total vitamin C content by rapid oxidative methods (6, 7). The retardation of oxidized flavors by deaeration and their stimulation in the presence of dissolved oxygen and vitamin C by the exposure of fresh milk to sunlight for a short period of time or by copper catalysis of the reaction (6, 7), indicates that oxygen plays an important part in the reaction which produces the oxidized flavors, and that the reaction is promoted by a catalyst. The prevention of the oxidized flavors by the complete depletion of the total vitamin C content of milk, irrespective of the oxygen and copper present, suggests that oxygen and copper, either alone or together, could not promote the reaction which produces the oxidized flavors (6, 7).

Thus, to develop the oxidized flavors in fresh milk, all components of the system must be present, namely, oxygen, vitamin C, catalysts such as copper, light or enzymes, oxidation-susceptible lipid fraction of the milk or, as recently reported, casein (13).

It long has been believed that vitamin C is an anti-oxidant, and that if it were increased in the milk, the development of the oxidized flavors would be prevented (1, 11, 12). Under certain conditions, vitamin C might exert a protective influence. However, the amounts of added ascorbic acid required for protection vary from sample to sample. Undoubtedly, this is due to the variation in the amounts of dissolved oxygen in the samples of milk. It has been shown that partial and quick oxidation of ascorbic acid in milk to dehydroascorbic acid, either by added hydrogen peroxide or by oxygen with light as a catalyst, brings the system more quickly to the point where the other reactions which produce the oxidized flavor in milk could be coupled to that involving ascorbic acid oxidation (6, 7). Consequently, the amount of ascorbic acid added to milk must be considerably in excess of that required to utilize all of the dissolved oxygen prior to

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the establishment of conditions favorable to the secondary reactions which result in the development of the oxidized flavor. The differences between samples of milk fortified with ascorbic acid in their abilities to resist the reaction which produces the oxidized flavor also could be attributed to variations in the ability of milk to promote ascorbic acid oxidation.

Ascorbic acid in milk is oxidized to dehydroascorbic acid which is a very unstable form of vitamin C and is readily destroyed by heat. Its accumulation in the milk is governed primarily by the rate of ascorbic acid oxidation. When dehydroascorbic acid is destroyed as rapidly as it is formed by the oxygenation of milk during pasteurization, the secondary reaction which produces the oxidized flavor is prevented. In this connection, it should be noted that the development of oxidized flavors in fresh milk apparently has nothing to do with the deterioration of milk fat, which is relatively stable. However, milk fat itself also undergoes oxidative deterioration in the presence of ascorbic acid, resulting in the development of metallic to fishy flavors. These flavors have been traced to oxidative processes which occur in the milk fat at the end of its storage life. Quite often they are accompanied by the extensive losses in vitamins A, E and carotene content of the milk fat. The susceptibility of milk fat to this type of deterioration depends on the type of product, the temperature of pasteurization and the conditions of storage. These factors were studied in some detail, and the data are presented to show that under certain conditions, namely, at the end of storage life of the milk fat, ascorbic acid plays an important part in the oxidative deterioration of fat, manifesting itself by the development of the objectionable oxidized flavors and the losses in the fat-soluble vitamins.

EXPERIMENTAL

In a recent study of the oxidation of vitamin A and its precursor, carotene, in milk fat (4, 5), it has been pointed out that the reemulsification test was found to be very useful in recognizing not only the flavor defects of milk fat, but also in detecting changes in the resistance of milk fat to oxidation, whether these changes are brought about during storage, by exposure to light, or any other factor. Consequently, in order to study the effects of different factors upon the ability of milk fat to resist deterioration in the presence of ascorbic acid, the following procedure was adopted.

At the end of various storage periods, milk fat, held either in the form of cream¹, butter¹ or pure fat¹, was re-emulsified in skim milk² depleted of its total vitamin C content by rapid oxidative method (7) to produce 4 per cent fat re-constituted milk. To aliquot portions of this milk, approximately 20 mg. of ascorbic acid and 0.1 mg. of copper per liter of milk were added, either alone or together. These portions of milk then were held at 0 to 5° C. up to 48 hours. Throughout the duration of the experiments, the samples were protected from

¹ Milk fats were obtained by churning of cream, oiling and centrifuging of butters prior to their use in the re-emulsification test, or prior to storage.

² In Club aluminum cream maker. The skim milk was depleted of its total vitamin C content by added H₂O₂ and the following pasteurization at 61.6° C (143° F) for 30 minutes (7).

TABLE 1

The effects of complete depletion of natural milk of its total vitamin C content by the oxygenation of milk during pasteurization upon the development of oxidized flavor in milk subsequently held at 0 to 5° C.

Held at 0-5° C.	Untreated control milk		(B) Oxygenated milk		Additions to (B) milk	
Days	Ascorbic acid	Flavor scores	Total vit. C	Flavor scores	Ascorbic acid	Flavor scores
	(mg./l.)		(mg./l.)		(mg./l.)	
0	12.3		00.0		20.8	
1	6.8	38 T	42	13.0	42
2	0.0	25 T	42	3.0	38
3	Ex. T.	42	0.0	25 T
Copper added—0.1 p.p.m.						
1	00.0	25 T	42	1.9	25 T
12		Ex. T.		42		Ex. T

light. They were scored for flavors; then the gravity cream was churned, and the butter obtained was centrifuged and the fat was analyzed for its fat soluble vitamin content. The judging was done on the basis of the score card recently revised by the American Dairy Science Association³. Vitamins A and E and peroxides were determined, using Koehen and Sherman (3), Quaife (10) and Volz and Gortner (14) methods, respectively.

It already has been remarked that when dehydroascorbic acid is destroyed as rapidly as it is formed, the secondary reaction which produces the oxidized flavors in milk is prevented. To test this point, a batch of fresh mixed milk was oxygenated during the pasteurization at 61.6° C. for 30 minutes. The oxygen was admitted in minute bubbles into milk undergoing treatment, using carborundum gas diffusion tubes.

TABLE 2

The effects of complete depletion of natural milk of its total vitamin C content by hydrogen peroxide and the following pasteurization and of added ascorbic acid and copper upon the development of oxidized flavors and the stability of fat soluble vitamins in milk subsequently held at 0 to 5° C.

Additions			Flavor scores of milk				At the end of 14 days holding period		
Ascor- bic acid	Cop- per	Ascor- bic acids	Hours at 0 to 5° C.				Per 100 g. fat		
			3	6	9	14	Carot.	Vit. A	Vit. E
(mg./ l.)	(mg./ l.)	(mg./ l.)					(μg.)	(μg.)	(μg.)
.....	40	40	40	40	438	421	2125
.....	0.1	40	40	40	40	433	419	2131
20.2	20.9	40 —	30MT	25MT	Ex.MT	432	401	2183
20.2	0.1	20.9	25MT	25MT	Ex.M.	Ex.M.	432	399	2071

² Ascorbic acid re-added at the end of a 6 day storage period.

³ Flavor scoring system: 40, no criticism; 35-40, acceptable to some consumers; 25, unsuitable for consumption. Symbols: Ex. = extremely; T. = tallowy; M. = metallic; F. = fishy; Oi. = oily.

The data presented in table 1 are rather conclusive in showing that the depletion of milk of its total vitamin C content by oxygenation prevents the development of the oxidized flavors, and that the oxidized flavors could be induced again by the addition of ascorbic acid to oxygenated milk. The results of this experiment are in agreement with the original one (6, 7).

The data of table 2 show that, although the oxidized flavors developed in samples of fresh milk containing added ascorbic acid prior to storage at 0 to 5° C., the vitamins A and E and carotenoid content of the fat remained practically unaffected. In this case, a sample of fresh mixed milk was first depleted of its total vitamin C content by hydrogen peroxide and the following pasteurization at 61.6° C. for 30 minutes.

The data presented in table 3 show the effects of the addition of ascorbic acid

TABLE 3

The effects of the addition of ascorbic acid and copper to reconstituted milks made of fresh skim milk depleted of its total vitamin C content or of skim milk powder and unstable fat (re-emulsification test) upon the development of oxidized flavors and losses of the fat soluble vitamins.

Reconstituted milk—4% fat							
Made of	Additions		Flavor scores of milk		Per 100 g. of fat		
	Ascorbic acid	Copper	Hours at 0 to 5° C.		Carotenoids	Vitamins	
			24	48		A	E
	(mg./l.)	(mg./l.)			(μg.)	(μg.)	(μg.)
fresh skim milk	Control fat				671	498	2062
1	40-oi	40-oi	640	441	1889
	20.7	Extremely		416	308	1234
	20.7	.1	metallic and fishy		395	269	1127
skim milk powder	40-	40-	599	438	2165
1	40-	40-	610	428	2018
	19.7	25 F	25 F	410	401	1074
	19.7	.1	Extremely metallic and fishy		380	305	1096

and copper to reconstituted milk (re-emulsification test) made of fresh skim milk depleted of its total vitamin C content, or of skim milk powder, and of fat susceptible to deterioration, upon the development of oxidized flavors and losses of the fat soluble vitamins. The results showed that extreme metallic to fishy flavors developed in samples of milk containing ascorbic acid added either alone or together with copper. The development of these flavors was also accompanied by considerable losses in vitamins A and E and carotenoid content of the fat.

These observations indicate, therefore, that the oxidized flavors in fresh milk are not associated with the milk fat, but with the unstable materials present either on the surface of the fat globules or in the plasma phase of the milk. This is clearly evident from a comparison of the data of tables 2 and 3.

Subsequently, it was thought of importance to obtain some idea concerning the effects of the following factors upon the development of the objectionable flavors and losses in the vitamins A and E and carotenoid content of the milk

fat in the re-emulsification test: the fat content of reconstituted milk, the amounts of added ascorbic acid, the exposure to light of fats susceptible and non-susceptible to oxidative deterioration, and the temperature of pasteurization of skim milk used in the test.

The data presented in table 4 show that in the re-emulsification test the development of the objectionable flavors was not retarded, and the losses in fat-soluble vitamins were not appreciably affected, either by the fat content or by the vitamin C content of the reconstituted milk products.

The data on the effect of the exposure of fat to light are presented in table 5. This experiment was prompted by the previous observations (4, 5), indicating

TABLE 4

The effects of the fat content of reconstituted milk (re-emulsification test) and of the amounts of subsequently added ascorbic acid upon the development of oxidized flavors and losses of the fat soluble vitamins

Type of fat (A)	Reconstituted milk made of skim milk and fat (A)								
	Fat content	Additions		Flavor scores		Per 100 g. fat			
		Ascorbic acid	Copper	hours at 0 to 5° C.		Carotenoids	Vitamins		Per-oxides
				3	24		A	E	
	(%)	(mg./l.)	(mg./l.)	(butm.)		(μg.)	(μg.)	(μg.)	(Milli-equiv.)
Suscept. to oxidative deterioration	Con.	fat	628	500	1909	0.516
	4.0	40-	40	612	482	1528	0.431
	4.01	40-	40	630	462	1737	0.397
	4.0	23.0	25T	Ex.F.	554	354	1084	0.368
	4.0	23.0	.1	Ex.FT	Ex.FT	401	268	785	0.497
	4.0	106.3	25T	Ex.FT	559	405	1265	0.371
	4.0	106.3	.1	Ex.FT	Ex.FT	331	245	721	1.116
	20.0	40-	40	594	480	2082	0.453
	20.01	40-	40	639	491	1714	0.511
	20.0	23.5	Ex.FT	Ex.FT	564	412	1187	0.505
	20.0	23.5	.1	Ex.FT	Ex.FT	493	328	970	0.880
	20.0	82.1	Ex.FM	Ex.FM	568	398	1403	0.354
	20.0	82.1	.1	Ex.FM	Ex.FM	420	359	1004	0.692

that vitamin A in the milk fat is readily photo-oxidized, whereas its precursor, carotene, remains unaffected. It was of interest, therefore, to find out also the effects of irradiation of stable and unstable fats upon the stability of tocopherols, and the susceptibility of fat to deterioration in the re-emulsification test. The irradiation experiment was performed following the same procedure as previously described (4, 5) with the exception that the temperature of the fat was maintained at 45 to 48° C. and the intensity of the light generated by the mercury vapor lamp at approximately 1400 foot-candles. The data in table 5, although not directly comparable, revealed that the susceptibility of fat to the foregoing type of deterioration depends primarily upon its ability to resist oxidation prior to irradiation, and to lesser degree upon the direct effect of irradiation. The data in table 5 also indicate that, irrespective of the ability of fat to

resist oxidation in the re-emulsification test, vitamin A was found to be photo-oxidized at a much faster rate than vitamin E, whereas the carotenoid content of the fat remained practically unchanged throughout the duration of the irradiation. Subsequently, it was thought of importance to obtain some idea concerning the effects of the temperature of pasteurization of skim milk used in the re-emulsification test upon the development of the objectionable flavors and losses in the vitamins A and E and the carotenoid content of the milk fat. For this reason, ascorbic acid and copper were added either alone or together to portions of reconstituted milks made of unstable fat and of fresh skim milks depleted of

TABLE 5

The effects of irradiation of fats susceptible and non-susceptible to deterioration with light generated by mercury vapor lamp upon the fat soluble vitamin content, and the susceptibility of fat to oxidative deterioration as determined by the re-emulsification test

Reconstituted milk made of skimmilk and fat (A)							
Minutes irradiat. at 45- 48° C.	Type of fat (A)	Additions		Flavor scores hours at 0 to 5° C.	Per 100 g. fat		
		Ascor- bic acid	Cop- per		Carot- enoids	Vitamins	
				6 & 48		A	E
Control	Suscept.	Control	fat	634	492	2047
	“1	40-Oi	602	416	1670
	“	20.0	fishy	387	316	1026
	“	20.0	.1	ex. F.	204	186	906
60 minutes	Suscept.	Control	fat	585	160	1540
	“1	40-Oi	553	167	1396
	“	20.0	fishy	443	116	1164
	“	20.0	.1	ex. F.	229	73	1134
Control	Non-sus- ceptible	Control	fat	1024	883	3917
		average all sam.		40	1019	872	3966
60 minutes	“	Control	fat	1026	308	3777
	“1	40-Oi	1015	240	3971
	“	24.3	39-Oi	1010	224	3840
	“	24.3	.1	35-OiMT	971	201	2809

their total vitamin C content by added hydrogen peroxide, and the following pasteurization at 61.6 and 76.6° C. for 30 minutes.

A comparison of the data in table 6 suggests the possibility that the inactivation of a catalyst-enzyme by heat primarily was responsible for the retardation of inter-action between ascorbic acid and fat or fat-soluble vitamins in the milk, which was made of skim milk pasteurized at 76.6° C.

Although the evidence presented in the preceding paragraphs definitely indicates that the oxidative deterioration of milk fat could be catalyzed in the presence of ascorbic acid, nevertheless, it was apparent that the reaction might take place only at the end of the storage life of fat. Since it seemed possible that the changes in the ability of milk fat to resist the foregoing type of deterioration would be affected by the type of product held, the temperature of pasteurization

TABLE 6

The effects of the temperature of pasteurization of skim milk used in the re-emulsification test upon the development of oxidized flavors and losses of the fat soluble vitamins.

Reconst. milk made of skim milk and unstable fat					Per 100 g. of fat			
Additions		Flavor scores			Carot- enoids	Vitamins		Perox- ides
Ascor- bic acid	Cop- per	Hours at 0 to 5° C.		A		E		
		½ & 24	48					
(mg./ l.)	(mg./ l.)	(Skm.) ^a	(Butm.) ^a	(μg.)	(μg.)	(μg.)	(milli- equiv.)	
Prior to re-emulsification				506	452	2431	0.196	
After re-emulsification in the skim milks:								
(I). pasteurized at 61.6° C.								
control		40	40	495	442	2197	.153	
.....	.1	40	40	483	432	2080	.202	
19.7	25F	25F	Ex. F.	399	1808	.356	
19.7	.1	Ex. F.	Ex. F.	F.	327	1294	.470	
(II). pasteurized at 76.6° C.								
control		40	40	473	436	2475	0.28	
.....	.1	40	40	501	444	2284	0.18	
22.1	40	40	30F	451	2121	0.22	
22.1	.1	40	35T	Ex. F.	449	1468	0.20	

^a Skm., gravity skim milk; Butm., buttermilk after the churning of gravity cream.

zation and the condition of storage, it was thought desirable to determine what effects these factors might have upon the storage life of fat as determined by the re-emulsification test.

For this reason, four lots of cream, butter and pure fat were prepared from mixed morning milks obtained from the Cornell University herd. Two portions of this milk were oxygenated continually during the pasteurization at 61.6 and 76.6° C. for 30 minutes. This was done to deplete the milk of the total vitamin C content prior to preparation and storage of the previously described milk products. The other two portions of the same milk were pasteurized at the same

TABLE 7

The effects of the depletion of milk of its total vitamin C content by oxygenation during the pasteurization, and the temperature of pasteurization upon the development of the tallowy flavor in cream during its storage at -17.7 to -16.1° C.

Milk		Flavor scores of cream and its buttermilk at the end of storage at -17.7 to -16.1° C.							
Treatment and Pasteurized at	Product	month							
		3	4	5	6	7	8	10	12
61.6° C. control	Cream	38T	39T	39T	40	39T	38T	38T	30T
	Buttermilk	38T	39T	40	40	39T	38T	40	35T
76.6° C. control	Cream	40	40	40	38T	25T	25T	25T	25T
	Buttermilk	38T	38T	39	ExTM	ExTM	ExTM	ExTM	ExTM
61.6 and 76.6° C. oxygenation	Cream and								
	Buttermilk	40	40	40	40	40	40	40	40

temperatures, but without the oxygenation treatment (tables 7 and 8). In another experiment, a portion of mixed milk was first depleted of its ascorbic acid content by adding hydrogen peroxide. The aliquot portions of each cream, separated from the control and ascorbic acid depleted milks, then were pasteurized at 61.6, 68.3, 71.1 and 76.6° C. for 30 minutes prior to churning and storage of cream, butter and pure fat (table 9). Throughout the duration of the experiments, the samples of cream and fat were held in tightly sealed glass containers protected from the light. The butter samples were shaped in cylindrical forms and wrapped directly in two thicknesses of tinfoil. The samples of cream, butter and pure fat subsequently were held at -17.7 to -16.1° C.

The data presented in table 7 show that the creams separated from milks

TABLE 8

The effects of the depletion of milk of its total vitamin C content by oxygenation during the pasteurization, the temperature of pasteurization and storage of cream, butter and fat at -17.7 to -16.1° C upon the susceptibility of fat to oxidative deterioration (storage life of fat) as determined by the re-emulsification test.

Milk		Product held	The end of the storage life of fat
Pasteurized at	Treatment		
(°C.)			(months)
61.6	control	cream	4
	oxygen.	cream	4 to 5
76.6	control	cream	5
	oxygen.	cream	6
61.6	control	butter	4 to 5
	oxygen.	butter	5 to 6
76.6	control	butter	not sensitive to deterioration at the end of 12 months
	oxygen.	butter	
- all temperatures	control & oxygen.	fat	"

Storage cream—56 to 58 per cent fat.

depleted of their total vitamin C content by oxygenation during pasteurization were found by judges to be perfect in flavor at the end of 12 months storage at -17.7 to -16.1° C. Moreover, it was found that the depletion of cream of its total vitamin C content by added hydrogen peroxide, and the following pasteurization at indicated temperatures resulted in the prevention of oxidized flavors, even at the end of 2 years storage at -17.7 to -16.1° C. It should be noted, however, that these creams developed a slightly "nutty" flavor at the surface, whereas the control samples of cream developed very strong talloxy flavor.

The data in tables 8 and 9 reveal that the temperature of pasteurization of milk or cream and the type of product held are the factors governing the susceptibility of fat to oxidative deterioration in the presence of added ascorbic acid. They also show that, irrespective of the temperature of pasteurization, the milk fat stored in the form of cream loses its resistance to oxidative deterioration at a much faster rate than the fat stored in the form of butter; and that only the

fat from butter churned from cream pasteurized at 71.1 and 76.6° C. and the pure fat were found to be non-susceptible to oxidation resulting in the development of the objectionable flavors when re-emulsified in skim milk at the end of 1- and 2-year storage periods.

The re-emulsification test was found to be extremely useful in determining the sensitivity of fat to oxidation when the fat was obtained from products which as yet have not shown any apparent changes either in their flavors or in their fat soluble vitamin content. This is clearly evident from a comparison of data of tables 7 and 8. It shows that the cream separated from oxygenated milk did not develop any "off" flavors during the 12-month holding period. This period, during which neither organoleptic nor chemical changes could be accurately determined, often is referred to as the storage life of fat or of a food product. Yet, in the presence of ascorbic acid, with or without copper, the fat undergoes deterioration which is manifested by the development of the objectionable flavors and the losses of fat-soluble vitamins. This would explain the development of the distasteful flavors in food products made of storage cream plus products containing vitamin C, such as fresh milk or lemon juice.

In conclusion, it is of importance to point out that the development of "off" flavors in milk, as a result of ascorbic acid oxidation with copper as a catalyst, might not necessarily be connected with the deterioration of milk lipids. This was evident from the fact that occasionally a flavor which is difficult to describe developed at the end of a 48-hour storage period in the skim milk used as a control sample in the re-emulsification test. This would be in line with the experimental results obtained by Thompson *et al.* (13).

Although this flavor might be present in the gravity skim milk obtained from the reconstituted milk, it seldom was detected in the gravity cream buttermilk. This suggests that either some factor, unknown at present, prevents the development of this flavor in the gravity cream, or that the substance responsible for it was denaturated during the churning of cream.

Usually no objectionable flavors develop in the gravity cream buttermilk when fat used in the re-emulsification test is stable. The metallic to fishy flavor developed in reconstituted milk made of oxidation-sensitive fat and containing ascorbic acid, either alone or with copper, quite often was intensified by the churning of gravity cream. This flavor does not develop in the presence of copper alone.

The oxidized flavors which developed in the control samples of milk products during their storage might be carried into the portion of reconstituted milk containing copper alone. The churning of gravity cream from such milk may not result necessarily in the intensification of this flavor.

SUMMARY

(1) It has been shown that ascorbic acid plays an important part in the oxidative deterioration of milk fat at the end of its storage life, as determined by the re-emulsification test, resulting in the development of objectionable

flavors and losses in vitamins A and E and the carotene content of the fat. The susceptibility of fat to this type of deterioration is determined primarily by the treatment of milk, the temperature of pasteurization, the type of product, the conditions of storage, and to a lesser extent upon the direct and immediate effect of the exposure to light.

(2) The exposure of pure fat to light generated by mercury vapor lamp (1400 foot-candles) slightly affects its vitamin E content. However, it lowers the resistance of vitamin E in the stable fat to deterioration as determined by the re-emulsification test.

(3) The re-emulsification test was found to be useful in determining the end of the storage life of fat when the fat was obtained from products which have not as yet shown any apparent changes in their flavors. This view is supported by the observations showing that the depletion of cream of its total vitamin C content, either by oxygenation, or by hydrogen peroxide, has prevented the development of the objectionable flavors for 12 and 24 months at -17.7 to -16.1° C., respectively. In the re-emulsification test, however, the fat obtained from oxygenated milk pasteurized up to 76.6° C. lost its ability to resist the foregoing type of deterioration at the end of 4 to 6 months of storage, depending upon the conditions of processing.

(4) Only the fat from butter churned from cream pasteurized at 71.1 and 76.6° C. and the pure fat retained their abilities to resist deterioration in the re-emulsification test at the end of two years storage at -17.7 to -16.1° C.

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LACTATING FACTORS FOR DAIRY COWS IN DRIED GRAPEFRUIT PEEL

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Recent reports by Huffman et al. (2, 3) have shown that dairy cows fed alfalfa alone declined abnormally in milk production. A marked increase was obtained by supplementing the alfalfa with corn silage (2) or beet pulp or corn gluten meal (3). These feeds were claimed to contain unknown milk stimulating factors. Because of the interest throughout the citrus-producing states in the use of dehydrated citrus by-products in dairy rations (1, 4, 5), experimental work was conducted to ascertain the value of dried grapefruit peel as a source of these milk stimulating factors.

EXPERIMENTAL PROCEDURE

Two sources of dried grapefruit peel¹ were used in the feeding trials. One was sun-dried in the open desert. No juice was removed from the peel previous to drying. The other was dried mechanically. Part of the juice was removed before dehydration.

Four pairs of cows were placed into two lots. One of each pair eventually received sun-dried peel and its mate mechanically dried peel. Each pair was identical in breed and nearly identical in period of lactation and age. Numbers 56, 57, 60 and 61, Guernseys, were fresh, 40, 42, 16 and 17 days, respectively, when placed on experiment; 158 and 159, Jerseys, 41 days; and 257 and 272, Holsteins, 50 and 33 days, respectively. Numbers 60, 61 and 257 were first-calf heifers and the remainder were second-calf heifers.

As soon as the cows were placed on experiment, they were fed first-cutting alfalfa hay ad libitum in dry lot until milk production markedly decreased. This period was of 6 weeks duration for 158, 159 and 272 and 9 weeks for the remaining five animals. At the end of this period, the alfalfa was supplemented with 2 lb. of dried grapefruit peel twice daily for each cow for 4- to 5-week periods. After this, the dried citrus was replaced with an equal amount of a grain mixture consisting of six parts barley, six parts wheat bran, two parts cottonseed meal and two parts beet pulp. After 4 weeks on this ration they were supplemented with oat pasture. Four lb. of the grain mixture furnished approximately 2.91 therms of energy and 4 lb. of the dried grapefruit peel approximately 2.98 therms.

RESULTS AND DISCUSSION

The milk production records are given in table 1. In every case there was a decline in milk production during the alfalfa feeding period. When supple-

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¹ We are indebted to Mr. E. G. Harrington, Exchange Orange Products Company, Tempe, Arizona, for generous supplies of dried grapefruit peel.

TABLE 1

Effect of dried grapefruit peel on milk production (Av. daily production during test week)

Feeding period	Sundried grapefruit peel fed			Mechanically dried grapefruit peel fed			
	Week of period	Guern- seys (av. of 56 & 60)	Jersey 159	Hol- stein 257	Guern- seys (av. of 57 & 61)	Jersey 158	Hol- stein 272
		(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)
Alfalfa alone (6-9 weeks)	First	30.7	25.4	36.7	31.1	33.3	43.4
	Last	20.9	15.7	26.7	19.7	19.0	19.4
Alfalfa + 4 lb. citrus daily (4-5 weeks)	First	22.2	16.7	28.7	20.9	18.5	23.4
	Last	22.4	15.6	29.2	20.8	18.5	20.9
	Av.	22.4	16.1	29.0	20.8	18.2	21.7
Alfalfa + 4 lb. grain mixture daily (4 weeks)	First	21.4	15.5	27.0	19.9	18.4	17.5
	Last	20.5	13.6	23.6	17.4	15.3	16.4
	Av.	20.4	15.0	25.4	19.0	16.9	17.5
Alfalfa + grain mixture + pasture (3 weeks)	First	21.8	13.4	25.3	20.7	17.5	
	Last	23.0	13.9	27.3	21.4	17.2	
	Av.	22.6	13.8	26.0	21.4	17.3	

ments of either source of dried grapefruit peel were given, there was a small but definite increase in milk production for seven of the eight cows. In the case of Jersey cow 158, the dried peel only lessened the decline caused by the alfalfa ration. However, milk production remained constant for this cow during the 5-week period in which the peel was fed. There was no significant difference between the results received with either of the two sources of dried grapefruit peel.

In order to eliminate the effect of energy in the dried grapefruit peel upon milk production, the dried peel was removed and an equal amount of grain mixture given. This grain mixture was approximate in energy to the dried grapefruit peel. Milk production definitely fell off in the first week of this period except for the two Jerseys. During this total period of 4 weeks, there was a definite decline in milk production for all the cows. This proved that the increase caused by the dried peel was not caused by added energy. When the cattle were

TABLE 2

Weight records of cows

Period of feeding	Weight of cow no.							
	56	60	159	257	57	61	158	272
	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)	(lb.)
Alfalfa alone								
At start	973	908	885	1133	942	780	843	1443
At end	982	853	873	1132	973	752	798	1490
End of alf. + 4 lb. citrus	985	878	890	1107	912	755	812	1527

placed on pasture there was a definite increase in milk production except for Jersey 159.

The weight records of the cows are given in table 2. During the alfalfa feeding period, there was no significant change in weight. Three of the cows gained weight, four lost weight and one remained the same. During the period of citrus feeding four of the cows definitely gained weight, two remained the same and two lost weight.

From the above results, it is apparent that both sundried and mechanically dried grapefruit peel contain the unknown milk-stimulating factors first demonstrated in certain feeds by Huffman et al. (2, 3). The grain mixture fed did not contain these factors in appreciable amounts, while oat pasture did. The findings give emphasis to the value of dehydrated citrus products in dairy rations.

SUMMARY

Four lb. daily of dried grapefruit peel added to an alfalfa hay ration increased milk production. An equal amount of a grain mixture did not maintain this increase. Supplementing a ration of alfalfa hay and concentrate mixture with oat pasture definitely increased milk production. It is concluded that dried grapefruit peel contains factors which stimulate milk production in dairy cows.

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THE NUTRITION OF THE NEWBORN DAIRY CALF III. THE RESPONSE TO A PHOTOLYZED MILK DIET

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A series of reports from the Illinois Agricultural Experiment Station recently have contributed significantly to the fundamental information on the nutrition of the very young calf. Wiese *et al.* (17) prepared a "synthetic milk" which resulted in normal growth when fed to calves from 48 hours to 12 weeks of age. When riboflavin was omitted from this "synthetic milk", deficiency symptoms developed in from 2 to 6 weeks (18). Since these experiments have shown that the very young calf requires a dietary source of riboflavin, it was of interest to study the effects of feeding natural milk in which a major portion of the riboflavin had been destroyed.

Numerous reports in the literature (4, 5, 7, 13, 14, 19) have shown that significant amounts of the riboflavin of milk are destroyed by exposure to sunlight. Preliminary work showed that under proper conditions of sunlight exposure sufficient quantities of the riboflavin in colostrum and milk could be destroyed to yield a product which was extremely low in this vitamin. Studies then were begun to determine the effect of feeding photolyzed colostrum and milk to newborn or very young calves.

EXPERIMENTAL

Four purebred, male, Guernsey calves were used in this experiment. Two of these calves (*A* and *B*) were taken from their dams at birth and placed in 6 × 6-foot wooden box stalls with wire mesh flooring. Calf *A* was bedded with straw. All subsequent calves were bedded on burlap sacks to eliminate the possibility of the ingestion of straw stimulating rumen synthesis of riboflavin. Calves *A* and *B* were fed for 3 days on colostrum which had been photolyzed previously, frozen and stored¹ for over 3 weeks. The two remaining calves, *C* and *R*, twin males, were fed normal colostrum from their dam for 72 hours. Following the colostrum feeding period all calves received, as an exclusive diet, photolyzed whole milk from the Ohio State University herd at the rate of 10 per cent of their body weight. The milk intake was reduced during periods of scouring. Calf *R* received, in addition to the milk, an average of 2.99 mg. of crystalline riboflavin per day. The calves were fed twice daily from nipple pails. The milk was heated to 37° C. before feeding, and the volume of milk fed was recorded. The calves were housed in a steam-heated calf barn and weighed at weekly intervals.

Riboflavin estimations were made on samples of the milk from each feeding, using a modification of the fluorimetric technique reported by Hand (3). Blood plasma vitamin A determinations were made periodically using the procedure of

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¹ Stored in frozen food containers generously provided by the Lily Tulip Cup Corporation, New York, N. Y.

Kimble (6). The method of Boyer *et al.* (1) was followed in estimating the vitamin A content of the milk and colostrum.

Approximately once each week, the experimental calves were placed on a 2 × 6-foot metabolism cage with a wire mesh floor and a 24-hour urine sample collected. A rubber feces-sack was attached to the calf to prevent contaminating the urine with feces. The urine was collected in a brown-glass bottle containing approximately 20 ml. of concentrated HCl. The final pH of the urine usually was about 1.5. The total volume was measured, and a representative sample was adjusted to pH 1.0, frozen and stored until assayed for riboflavin. The storage time averaged about 1 week, but in one case was as long as 6 weeks. Riboflavin determinations were made on the stored urine, using the fluorimetric technique of Slater and Morell (12). Recent work by these authors (11) has shown that human urine stored at a low temperature and a low pH gave lower riboflavin values upon analysis than when stored at a higher pH and at room temperature. No major discrepancies were observed in our results, however.

Since sunlight could not be depended upon as a constant source of photolyzing

TABLE 1

The effects of the photolysis treatment on the vitamin A, carotene and riboflavin of milk

Milk sample no. and treatment	Vitamin A		Carotene		Riboflavin	
	γ/liter	% loss	γ/liter	% loss	mg./liter	% loss
1. None	264		289		1.42	
1. Photolyzed	46	82.5	252	12.8	0.05	96.5
2. None	203		321		1.54	
2. Photolyzed	49	75.7	287	10.6	0.06	96.1

energy, a DH-1 400 watt mercury vapor lamp² was secured. This lamp emitted rays longer than 3000 Å, thereby eliminating excessive irradiation at the wave length of vitamin D activation. The lamp was used in conjunction with a highly polished parabolic aluminum reflector, and the rays were directed at a 2-gallon rectangular museum jar containing the milk. The milk was agitated slowly by continuous stirring. About 96 per cent of the riboflavin was destroyed by a 3-hour exposure. During the photolyzing period, the milk usually was allowed to reach a temperature of at least 60° C. for one-half hour as a bacteria-control measure.

The photolyzed milk was chalky white in appearance and had a pronounced "sunlight flavor". The milk seldom was over 36 hours old when fed and in all cases was kept under continuous refrigeration except during the period of light treatment. Vitamin A and carotene, as well as riboflavin, were destroyed in appreciable quantities, as shown in table 1. It will be noted that a treatment which destroyed over 96 per cent of the riboflavin also reduced the vitamin A by 75 to 80 per cent and the carotene by 10 to 12 per cent of their original quantities.

The biological inadequacy of the photolyzed milk was verified by a rat growth

² Purchased from the Westinghouse Electric Corporation.

test. Twenty weanling albino rats were divided equally into four groups as to size and litter. An attempt was made to distribute them equally as to sex, but one group (group 4) had three males and two females while all other groups had three females and two males. The groups were fed ad libitum the diets, as shown

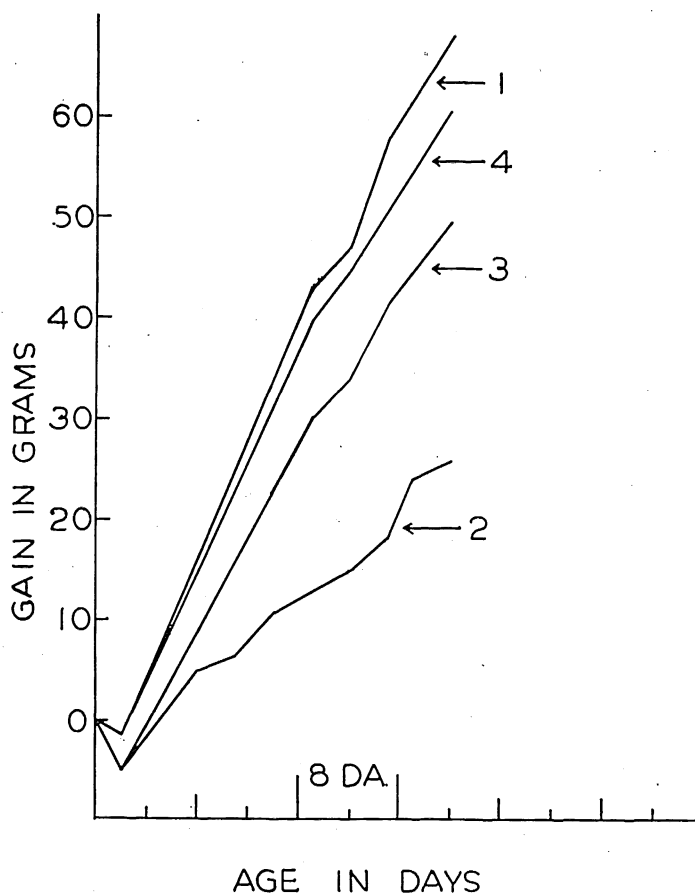


FIG. 1 Growth curves of rats fed the various mineralized milk diets:

Group	Diet	Grams gain/100 ml. consumed
1	Normal milk	6.10
2	Photolyzed milk	3.91
3	Photolyzed milk + riboflavin	5.22
4	Photolyzed milk + riboflavin + codliver oil	6.06

One drop of a mineral mixture solution containing 500 mg. each of ferric citrate, manganous sulphate and copper sulphate in 500 ml. was added to each feeding.

Riboflavin in sufficient quantity to approximate that of normal milk was added to the milk of groups 3 and 4.

in the legend of figure 1. The respective rates of growth are recorded graphically (fig. 1). The gain per 100 ml. of milk consumed also is shown. Obviously the photolyzed milk had a low biological value for growth, and when such milk was

supplemented with both riboflavin and vitamin A the response was similar to that obtained from normal milk. The amount of gain per 100 ml. of milk consumed was almost identical (6.10 g. and 6.06 g., respectively, for Groups 1 and 4) indicating that the difference in the rates of growth was not due to a difference in nutritive value of the milk consumed but to a difference in total consumption. As the photolyzed milk had a pronounced "sunlight flavor", it is probable that the palatability of the photolyzed milk was lowered, and this in turn reduced the consumption.

Because of the low vitamin A content of the photolyzed milk, supplemental vitamin A was fed to each calf. The vitamin A oil was mixed with soya-lecithin

TABLE 2
Average daily riboflavin intake in milligrams

Age	Riboflavin intake values for Calf						
	A	B	C	R			
				In photo- lyzed milk	Ribo- flavin added	Total intake	If fed normal milk ^c
	(γ)	(γ)	(γ)	(γ)	(γ)	(γ)	(γ)
1 day	1.24	2.05	9 ^b	9 ^b	9 ^b
2 days	0.82	0.71	9 ^b	9 ^b	9 ^b
3 "	0.64	0.29	9 ^b	9 ^b	9 ^b
4-7 days	0.23	0.18	0.15	0.15	2.63	2.78	3.69
2 wks.	0.22	0.13	0.11	0.10	2.46	2.56	3.45
3 "	0.21	0.07	0.13	0.13	2.71	2.84	3.80
4 "	0.23	0.13	0.14	0.14	2.59	2.73	3.63
5 "	0.15	0.11	0.12	0.16	2.90	3.06	4.06
6 "	0.15	0.10 ^a	0.17	0.18	3.01	3.19	4.22
7 "	0.17	0.15 ^a	0.19	0.22	3.37	3.59	4.72
8 "	0.17	0.17 ^a	0.15	0.17	3.61	3.78	5.05
9 "	0.12	0.20	0.10	0.18	3.47	3.65	4.86
10 "	0.22	0.23					
11 "	0.15	0.19					
12 "		0.16					
Average, 4th day to term- ination	0.18	0.15	0.14	0.16	2.99	3.13	4.16

^a Calf B was fed 2 mg./day in addition to that present in the milk.

^b Calves were fed normal colostrum from their dams. No record was kept of riboflavin intake.

^c Calculated on the basis of the calf's intake of normal milk containing 1.4 mg. of riboflavin per liter.

(2) and a small amount of photolyzed milk in a Waring Blender. The amount of vitamin A fed varied from 5,000 to 25,000 I.U. daily.

The average daily riboflavin intake for each calf is recorded in table 2. During the colostrum feeding period, calves A and B received 2.7 and 3.0 mg. of riboflavin, respectively. This is less than 11 per cent of the amount found in an equal quantity of average Guernsey colostrum (16). Since calves A and R were not assigned to the experiment until 3 days of age, their riboflavin intake during the colostrum feeding period is unknown. From the fourth day to the termination of the experiment the daily riboflavin intake averaged about 4 per

cent of a normal intake with the exception of calf *R*. Assuming that normal milk contains about 1.4 mg./liter (an average found in our laboratory), this latter calf received approximately 72 per cent of the amount normally found in the milk prior to photolysis.

The growth rate of the calves is shown graphically in figure 2, with the Ragsdale standard (8) included for comparison. The growth of calf *A* was normal and uneventful for the first 4 weeks, except for a brief period of scours during the first week which responded to sulfathaladine medication. From the fourth week, the calf suffered from intermittent scours which showed little improvement on sulfathaladine treatment. The quantity of milk fed was reduced at each

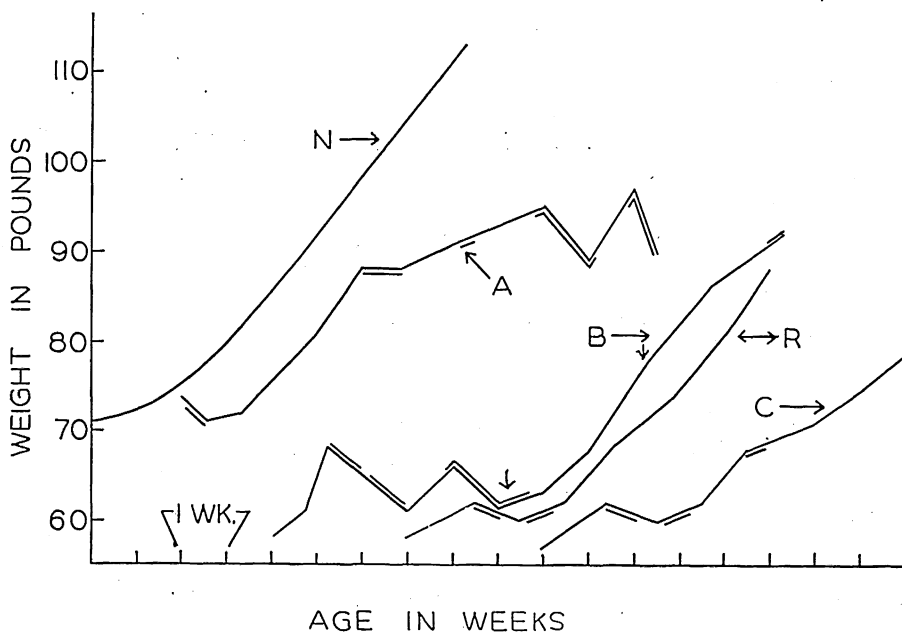


FIG. 2 Growth curves of calves used in this experiment. Double line indicates period of diarrhea. (N=Ragsdale standard, other letters refer to calves as designated in the text).

period of scours, and the calf never was able to consume the full allotment without scouring from the fourth week. An increasingly unthrifty condition developed, and obvious symptoms of riboflavin deficiency appeared at about 6 weeks. There were periods of excessive salivation and lacrimation. The haircoat became rough; there was evidence of a mild dry scaly dermatitis and a generalized alopecia which was more pronounced about the eyes and muzzle. A blood plasma ascorbic acid determination at 10 weeks showed 0.42 mg. per 100 ml., which was considered to be within the normal range. Periodic blood plasma vitamin A determinations (see table 3) showed normal levels, with the possible exception of the last two, which were taken towards the end of the experiment. These low values were considered to result from, rather than to be the cause of, the poor physical condition. Riboflavin excretions are shown in table 4. It will be noted that they remained low throughout the experiment.

TABLE 3
Blood plasma vitamin A of the calves used in the experiment

Age	Blood plasma vitamin A values for Calf:				
	A	B	C	R	Normal ^c
	(γ /100 ml.)	(γ /100 ml.)	(γ /100 ml.)	(γ /100 ml.)	(γ /100 ml.)
Birth	0.41	3.3
2 day	4.86
3 "	15.86	11.43	13.36	14.7
4 "	4.99
1 wk.	19.30	13.1
2 "	13.40	12.3
3 "	12.77	9.87	11.78	12.16	10.1
4 "	9.0
5 "	12.45	10.75	10.75
6 "	10.90	8.90	8.99
7 "	12.60	8.79 ^a	12.15 ^a
8 "	9.49	13.45	9.7 ^d
9 "	13.8	6.33 ^b
10 "	8.88	15.0
11 "	9.96	12.1
12 "	12.9 ^d

^a Vitamin A supplement was increased from 5,000 I.U. per day to 10,000 I.U. per day.

^b Level determined 3 days after preceding determination while animal was in a state of collapse.

^c Based on values obtained in this laboratory from nine Guernsey calves.

^d Based on average values from seven calves.

Calf A was sacrificed at 10.5 weeks of age and a gross post mortem examination showed marked catarrhal enteritis, mild edema of the cerebrum and "white spotted" kidney. One cornea was pebbled in appearance similar to that reported by Street et al. in the riboflavin deficient dog (15). The rumen contained approximately 1 liter of macerated straw and fluid. The rumen fluid contained 0.22 mg. per liter of riboflavin, which is about three times the concentration found in the milk consumed. Incubation of a portion of the rumen contents for 16 hours

TABLE 4
Urinary excretion of riboflavin by the calves used in the experiment

Age	Urinary riboflavin excretion by Calf:			
	A	B	C	R
	(mg./day)	(mg./day)	(mg./day)	(mg./day)
24 hr.	0.30
36 hr.	0.10
1 wk.	0.05	0.86	0.76
2 wk.	0.01	0.01
3 wk.	0.01
4 wk.	0.07	0.02	0.44
5 wk.	0.01
6 wk.	0.03	0.05	0.60
7 wk.	0.05 ^a	0.05	0.38
8 wk.	0.03	0.09 ^a	0.64
9 wk.	0.04
10 wk.	0.03
11 wk.	0.03

^a Received 2 mg. of crystalline riboflavin added to its diet during this period.

at 37° C. resulted in a 33 per cent increase in riboflavin concentration. These findings are considered as evidence of some microbiological synthesis, although insufficient in amount to fully protect the calf.

Calf *B* grew normally (fig. 2) for 1.5 weeks. Intermittent scours then resulted in poor growth until it was 5.5 weeks of age. At this time, the calf had been scouring for 9 days and was extremely unthrifty. It had a rough haircoat, a scaly dermatitis and mild alopecia which was more pronounced around the head. When a reduction in milk together with sulfathaladine treatment failed to improve the diarrhea, 2 mg. of crystalline riboflavin per day were added to the photolyzed milk for a period of 3 weeks. This addition to the diet resulted in the cessation of scours within 3 days, marked improvement in general appearance including the growth of new hair and the cessation of the excessive salivation and lacrimation. The amount of riboflavin excreted increased as shown in table 4. Growth was resumed almost immediately, and for the period of supplementary feeding it compares favorably with the Ragsdale standard. The

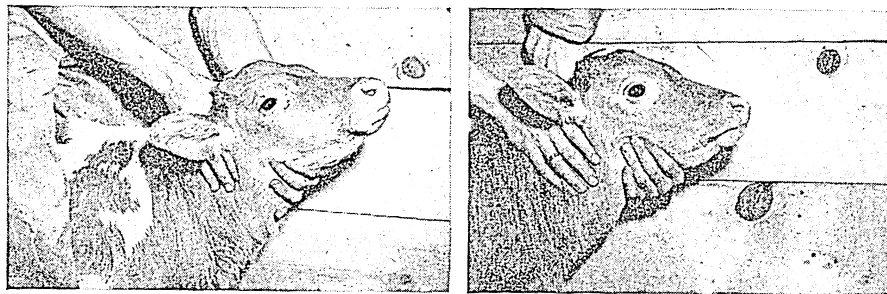


FIG. 3 Left, Calf *B* at 35 days of age, 3 days before 2 mg. of riboflavin were added to its daily diet. Note the excessive salivation. Right, The same calf at the conclusion of the 3 week supplement feeding period.

improvement of the deficiency symptoms following riboflavin therapy is shown in figure 3. Blood plasma vitamin A was within the normal range throughout the experiment, as shown in table 3. The blood plasma ascorbic acid at 9 weeks of age was 0.44 mg. per 100 ml. and was considered to be normal. Post mortem examination showed mild catarrhal enteritis as the only gross pathology.

The twin calves (*C* and *R*) differed by 1 lb. in weight at birth and critical body measurements were identical. They responded in an identical manner for the first 4.5 weeks, (fig. 1). At this point, the growth rate of calf *C* became slower while calf *R*, which was receiving a continuous daily riboflavin supplement, grew at a rate which approached the Ragsdale standard. No difference between these calves could be detected until after 4.5 weeks of age. From this time on, calf *C* was distinctly more lethargic, showed increased salivation and lacrimation, a dry scaly dermatitis and alopecia, particularly around the eyes and base of the ears. Calf *R* appeared completely normal.

About 2 weeks before calf *C* was sacrificed, it had marked difficulty in swallowing. It had to release the nipple several times during each feeding to clear its throat. About 48 hours prior to exsanguination it refused its feed. Thirty

hours later it was found in a state of acute collapse. It could not walk but was able to stand for a few seconds. A thin, watery salivation was excessive. The calf failed to respond to an intravenous injection of 500 ml. of "Intrigel"³. Five mg. of riboflavin in physiological saline were injected intravenously 5 hours later. The calf was still alive 12 hours later but not noticeably improved in condition. At this point, the calf was destroyed and tissues obtained for microscopic study. The gross pathology consisted of marked catarrhal enteritis, a mild edema of the lungs and a few scattered spots on the kidney. No evidence of pneumonia was observed.

No gross pathology was found in calf *R* following exsanguination approximately 3 weeks later.

At 6 weeks of age the vitamin A supplement of both calves *C* and *R* was increased from 5,000 to 10,000 I.U. per day. It will be noted from table 3 that calf *C* failed to respond. This is further evidence that the low blood plasma vitamin A resulted from the riboflavin deficiency syndrome, probably the severe enteritis. Blood plasma ascorbic acid was within the normal range in both calves at 6 weeks of age (0.45–0.46 mg./100 ml.). Calf *R* gained 19.5 lb. per 100 l. of milk consumed, while calf *C* gained only 15.7 lb. on a similar amount. Thus, the difference in rate of growth can not be attributed entirely to the difference in the amount of milk consumed.

The residual effect of the high riboflavin intake during the colostrum feeding period is shown by a relatively high excretion in both calves at 1 week of age. Following this, the excretion in calf *C* decreased to a low level (see table 4) while that of calf *R* was maintained at a level 7 to 12 times higher for the remainder of the experiment. A single 24-hour urine collection from a 6 weeks old Guernsey calf which had been fed milk, hay and grain in the usual manner showed an excretion of 2.86 mg. per day. No clearcut lesions of the lips, gums or eyes, with the exception of one pebbled cornea, were observed in any of the calves in this experiment.

The process of photolysis no doubt destroyed other vitamins, particularly ascorbic acid and pyridoxine. The partial or complete destruction of these was not considered as a complicating factor in these experiments for the following reasons: It has been shown that calves do not require a dietary source of ascorbic acid (17). A marked poikilocytosis which responded to pyridoxine treatment has been described in adult cattle (9, 10). A few poikilocytes were found in the blood of only one calf in this experiment (calf *B*), and the number was no larger than one might expect to find in the mild anemia from milk feeding.

An approximate riboflavin requirement for a very young calf was determined from the data presented. Calf *R* made normal response to a diet of 3.13 mg. per day and grew from a weight of 58 lb. to 89 lb. or a mean of approximately 70 lb. Therefore, this calf responded normally to an average daily intake of 94 γ per kg. of body weight. Calf *B* responded at 5.5 weeks of age to an average daily

³ (8 g. of pyrogen-free gelatin per 100 ml. of 0.85 per cent saline) manufactured by Fort Dodge Laboratories Inc., Fort Dodge, Ia.

riboflavin intake of 2.15 mg. or, based on the weight of the calf (61 lb.), 77.4 γ per kg. of body weight. Since in vitamin deficiency diseases the amount required to cure the disease is usually larger than the amount required as a preventive, it seems logical that the growth requirement is even less than 77.4 γ per kg. of body weight. If calves were fed normal milk containing 1.4 mg. per liter at the rate of 10 per cent of the body weight, they would receive 140 γ per kg. which is almost twice the amount that was necessary to cure the symptoms of calf *B*. Thus, it seems that the possibility of a riboflavin deficiency developing during the milk feeding period is slight.

SUMMARY AND CONCLUSION

Approximately 96 per cent of the riboflavin in milk fed was destroyed by exposure to the radiations of a 400 W. mercury vapor lamp emitting light of wave lengths longer than 3,000 Å. Appreciable quantities of vitamin A and carotene also were destroyed. Four male Guernsey calves were fed this treated milk supplemented with adequate vitamin A. One of these calves also received approximately 2.99 mg. of added riboflavin daily.

Riboflavin deficiency symptoms consisted of erratic growth, intermittent diarrhea, a dry scaly dermatitis, alopecia, particularly about the head, periodic excessive salivation and lacrimation and in the acute stages, dysphagia and a peculiar collapse syndrome (one calf). Post mortem examinations showed evidence of catarrhal enteritis, mild edema of the lungs, (the collapse victim), pebbled cornea (one calf), mild edema of the cerebrum (one calf) and abnormalities of the kidney in two cases. These calves were extremely unthrifty. The addition of 2 mg. of riboflavin daily to the diet of one of these calves resulted in a prompt cessation of diarrhea, resumption of growth and a marked improvement in general appearance, including the growth of new hair. No other lesions of the lips or mouth or abnormalities of the eyes were noted.

The performance of the calf receiving 2.99 mg. of added riboflavin from the start was uneventful and approached the Ragsdale standard of growth.

Blood vitamin A and ascorbic acid levels were normal in all four calves. The urinary excretion of riboflavin varied from 0.01 to 0.06 mg. per day for calves receiving the treated milk with no added riboflavin and 0.38 to 0.64 mg. per day for the calf which received added riboflavin throughout the experiment.

The limited data of this experiment indicate that the minimum daily riboflavin requirement of the very young calf is somewhat less than 75 γ per kg. of body weight. The possibility of riboflavin deficiency during the milk feeding period is remote.

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SOME CHANGES IN DRY WHOLE MILK DURING STORAGE¹

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Numerous changes other than the oxidation of the lipides are known to occur in dry whole milk during storage. Those in flavor are most apparent, although changes which can be measured more objectively may be important and would be most valuable as indices of deterioration if they could be shown to be associated with flavor.

The general factors influencing the development of a tallowy flavor in dry whole milk now are well known. Actual tallowiness does not develop unless more than 0.3 ml. of oxygen per gram of powder is available, although Lea *et al.* (15) and Coulter (4) have shown that lower oxygen levels improve the keeping quality. Flavor deterioration is most rapid during the initial period of storage and once the oxygen has been exhausted from the free-space gas, low-moisture powder becomes almost stable in quality at normal storage temperatures (4, 15).

Stale and allied flavors in dry whole milk have never been exactly defined. Supplee (20), Tillmans and Strohecker (22), Hunziker (12) and many others in the industry have recognized stale, musty or gluey flavors believed to be associated with the protein fraction of the milk and have presented evidence that these flavors become more marked in high moisture powders. Lea *et al.* (15) noted the appearance, in powder stored at 47 and 37° C. for some time, of a flavor variously described as "heated," "burnt," "scorched" or "cooked". This flavor was considered to consist of two components, (a) a "burnt" or "caramel" taste associated with the protein or carbohydrate, and (b) a "butter-toffee" flavor associated with the fat.

Loss of solubility and browning accompany staling (12, 15). Doob *et al.* (8) have published extensive data on the influence of moisture on the browning of dried whey and skimmilk. Tarassuk and Jack (21) have reported on the browning reaction in whole milk powder and ice cream powder since this investigation was completed. McCreary (16) has noted a decrease in soluble lactose in dried milk stored for a year at room temperature.

Lea *et al.* (15) noted the disappearance of oxygen in sealed cans of both skim and whole milk powders and the production of carbon dioxide.

Chapman and McFarlane (3) developed a method for determining acid ferri-cyanide reducing substances in dry milk and reported that they increased in dry whole milk stored in contact with the atmosphere. Modifications of the method and indications as to the source of the reducing materials have been presented by Lea (14) and Crowe *et al.* (6).

The production of fluorescent materials has been demonstrated to be asso-

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ciated with the deterioration of certain foods. Jenness and Coulter (13) have reviewed the literature on this subject and have suggested a method for evaluating the fluorescence characteristics of dry milk based on successive extractions with (a) 67 per cent acetone, (b) 20:80 acetone—ether and (c) 10 per cent KCl, and determinations of the blue fluorescence of each solution.

The browning reaction recently has been reviewed by Cantor *et al.* (2). Maillard, in the original publications concerning the reaction which now bears his name (Maillard or browning reaction), reported that the evolution of carbon dioxide, production of water (12 moles of water for every mole of carbon dioxide) and the formation of water-insoluble products accompanied the browning. Maillard also concluded that atmospheric oxidation played no part in the reaction.

Pearce (18) compared the suitability of a number of objective tests with subjective scores of palatability, and concluded that palatability was the most precise. This conclusion is not surprising considering the complexity of the system and the deficiencies in information concerning the deteriorative changes which occur.

The purpose of this research was to study changes which occur in dry whole milk during storage other than those associated with the lipides, and to secure some information on the effects of moisture, oxygen and temperature on these changes.

MATERIALS

Some observations were made using simplified systems as described by Jenness and Coulter (13). Briefly, these were prepared from acid-precipitated casein, dialyzed-milk serum protein, filtered milk fat, a concentrate of fat-globule "membrane" from washed cream and commercial samples of lactose and ascorbic acid and were dried from the frozen state.

Unless otherwise noted the experimental work to be reported on dry whole milk involved two large lots of powder, one (lot 384) of commercial origin and the other (lot K33) manufactured in an experimental spray drier in the University laboratories, using a standard procedure comparable to commercial practice. Portions of each lot were adjusted by exposure to humid air to secure samples varying in moisture content from 2.0 to about 5.0 per cent. The samples were canned and nitrogen-packed to secure oxygen levels ranging from approximately 1.0 to 6.0 per cent. Samples at each moisture and oxygen level were stored at 20, 37 and 60° C. The samples of the commercial powder stored at 60° C. were lost due to failure of the heat regulator of the incubator. The samples stored at 60° C. were analyzed at 10-day intervals up to 50 days, those at 37° C. at 4-week intervals up to 16 or 20 weeks and those at 20° C. at 8-week intervals up to 32 weeks. Some data were secured on other lots of dry whole milk manufactured in the experimental drier.

EXPERIMENTAL

Change in flavor on storage. Over 1,000 samples of dry whole milk representing both commercial and experimental production were scored by a selected

panel of five judges. A flavor described as "burnt feathers" was recognized in 84 samples. In only 9 instances was this criticism used in describing the flavor of dry whole milk containing less than 2.0 per cent moisture. Unless obscured by tallowiness or the caramelized flavor of powder which had become "brown," it was recognized in virtually all samples of stored powder containing more than 3.0 per cent moisture. Although it appeared more rapidly at the higher temperature, it was recognized in dry whole milk stored at 20, 37 and 60° C.

The flavors of the stored powders in lots 384 and K33 are typical. After 10 days at 60° C. the powder at the two highest moisture levels (3.81 and 4.49 initial) was brown and had the characteristic caramelized flavor. After 8 weeks at 37° C. and 16 weeks at 20° C., all samples, regardless of oxygen level, which contained more than 2.5 per cent moisture, were criticized as having the burnt feathers flavor. At the lower moisture levels, the samples were criticized as stale and finally tallowy at oxygen levels above 4.0 per cent.

In an attempt to determine the source of the burnt feathers flavor, frozen-dried simplified systems were prepared consisting of calcium phosphocaseinate with and without the addition of one or more of the following constituents: lactose, butterfat, serum protein, fat-globule membrane material and ascorbic acid. These were stored at 37° C. over sulfuric acid-water mixtures to obtain vapor pressures comparable with dry whole milk of low (2.5 per cent) and high (5.0 per cent) moisture. The calcium phosphocaseinate systems remained virtually unchanged in flavor. Those containing the phosphocaseinate and lactose, either with or without the addition of the other constituents, acquired the characteristic burnt feathers odor at the higher vapor pressure, and a characteristic stale flavor at the lower vapor pressure. The burnt feathers flavor therefore appears to be associated with lactose-protein changes and appears in high-moisture but not in low-moisture powder. The characteristic stale flavor that develops in normal dry whole milk probably is a composite of flavors resulting from lactose-protein changes and oxidation of the lipids. The flavor designated in this study as burnt feathers is probably the same as that described by others as "gluey."

Relation of moisture content to flavor deterioration. The importance of moisture content to the overall deterioration of gas-packed dry whole milk is shown graphically in figure 1. The samples involved are those gas-packed at the two lowest oxygen levels (below 2.0 per cent) from lots 384 and K33. Although, as shown by Lea *et al.* (15) and Coulter (4), the rate of loss in score of adequately gas-packed dry whole milk is not a straight line function of time but decreases with time, the total loss in score at any time interval can be used as an index of the overall rate of deterioration. The average weekly loss in score was computed from the difference between the original (fresh) score and the score after storage for the longest period of time, or in the case of the higher moisture samples at the higher storage temperatures, from the difference between original score and that at the last period at which an actual score was given. The logarithms of the weekly loss in score at 20, 37, and 60° C. with

one lot and at 20 and 37° C. with the other lot are shown in figure 1 plotted against the moisture content. The rate of loss in score appears to increase logarithmically with increase in the moisture content. Considerable variability in the rates of loss in score between the different lots of powder at any given moisture content and temperature is evident. It is realized that since the rate of loss in score of dry whole milk is not a straight line function of time, the comparisons made are empirical; however, the conclusions are believed to be

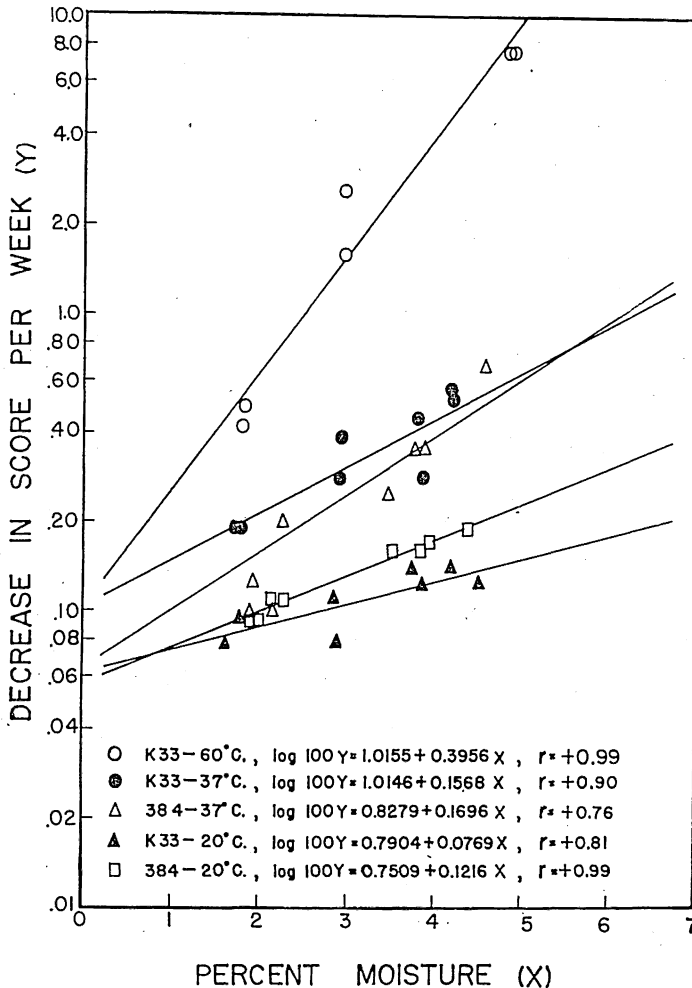


FIG. 1. The relationship of moisture content of dry whole milk to rate of loss in flavor score.

valid when dealing with adequately gas-packed dry whole milk. Holm and Greenbank (11) have shown that the minimum moisture content is not optimal in preventing tallowiness of air-packed powder. Gyorgy *et al.* (9) and Williamson (23) have presented evidence indicating that certain antioxidants require moisture for effectiveness.

Acid ferricyanide reduction. The total and non-protein acid ferricyanide

reducing capacity was determined using the method described by Crowe *et al.* (6). Initial trials were made using 9 lots (162 samples) of spray-dried whole milk produced on the experimental drier. The moisture content of the lots was varied within the range of 1.32 to 4.78 per cent. In some instances moisture variation was secured by drying to different levels, in others by exposing portions of the powder in thin layers to a humid atmosphere. Part of the powder in each lot was packed in air in no. 2 cans, and part was gas-packed in

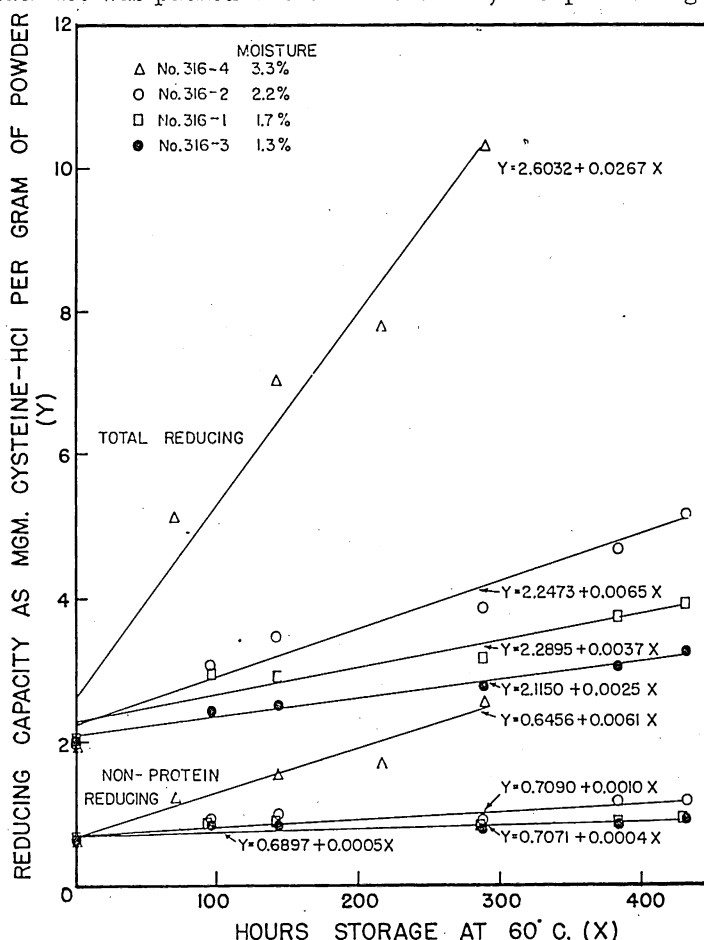


FIG. 2. Change in acid ferricyanide reducing capacity of dry whole milk stored at 60° C.

nitrogen to secure an oxygen level of less than 2 per cent. The powder was stored at $60 \pm 1^\circ$ C. and samples for analysis removed at intervals up to 432 hours.

The data for one group of samples which are graphed in figure 2 show an increase in both total acid ferricyanide reducing substances and non-protein acid ferricyanide reducing substances with time of storage at 60° C. Since the relationship appears to be essentially linear, the regression line for each powder was computed. The rate of production of acid ferricyanide reducing sub-

stances increases with increase in the moisture content of the powder. The data for the other samples (not presented) indicate a similar relationship.

To show in a more striking manner the relationship of the moisture content of the powder to the production of acid ferricyanide reducing substances, the log slopes of the regression lines were plotted against the average moisture contents of the samples (fig. 3). An increase in the moisture content of the

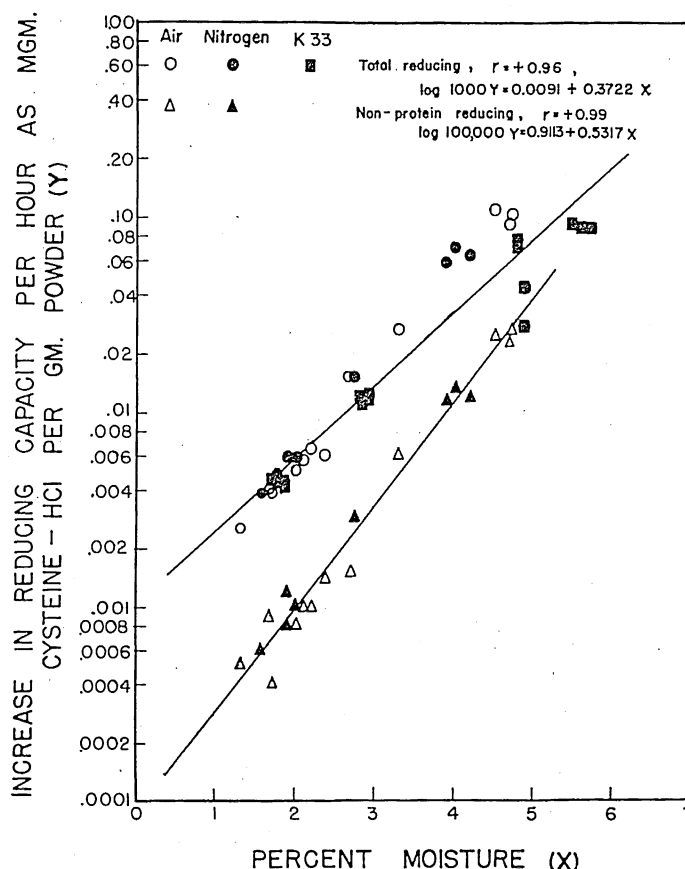


FIG. 3. The relationship of moisture content of dry whole milk to rate of increase in acid ferricyanide reducing capacity during storage at 60° C.

powder is accompanied by a logarithmic increase in the rates of production of both total and non-protein acid ferricyanide reducing substances. The data clearly indicate that the oxygen content of the atmosphere in contact with the samples is without effect on the rate of production of acid ferricyanide reducing substances.

The above observations were supplemented by data secured on lots 384 and K33. The results for the changes in the acid ferricyanide reducing capacity of the samples stored at 60° C. are plotted in figure 4. Since the oxygen level was without effect on the production of acid ferricyanide reducing substances, the

values shown for each moisture level are averages for the samples at the four oxygen levels.

The previously demonstrated linearity in the rate of production of acid ferricyanide reducing substances in dry whole milk stored at 60° C. is not maintained, particularly in the high moisture powders, on storage for longer than 10 days. In fact, a maximum may be reached followed by an actual reduction in the acid ferricyanide reducing capacity. This indicates a secondary reaction involving the non-oxidative utilization of the acid ferricyanide reducing substances.

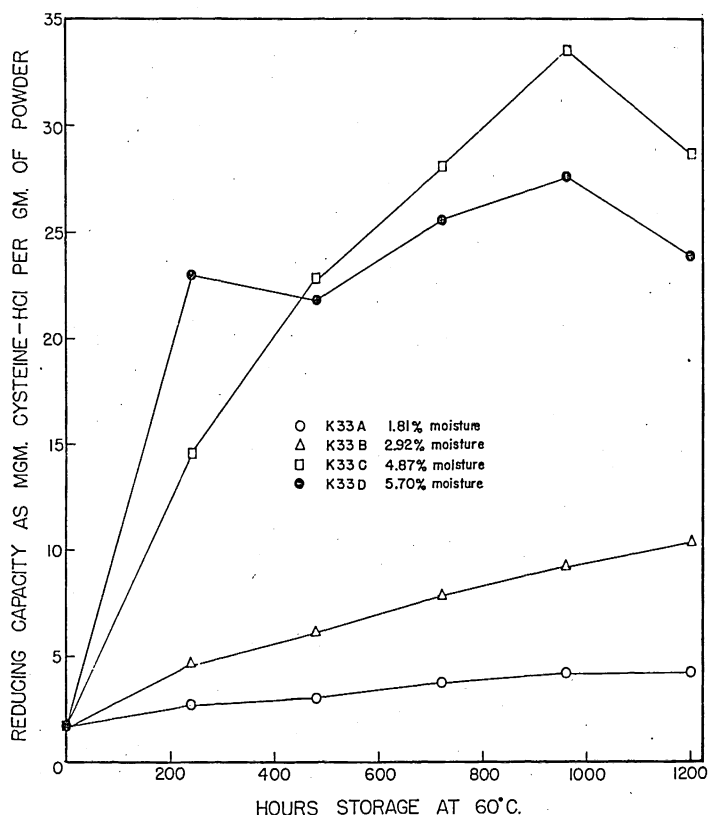


Fig. 4. Change in acid ferricyanide reducing capacity of dry whole milk stored at 60° C.

The rate of production of acid ferricyanide reducing substances in each sample was computed from the initial and 10-day values. These data plotted against moisture content in figure 3 confirm the relationship between the moisture content of the powder and the log of the rate of production of acid ferricyanide reducing substances during the initial storage period.

The data on the reducing capacity of the samples stored at 37 and 20° C. are summarized in tables 1 and 2. Since the oxygen level appears to have been without effect on the reducing capacity, only the average values for the samples at any given moisture level are shown. The initial reducing capacity of the

TABLE 1

Effect of storage at 37° C. on the acid ferricyanide reducing capacity of dry whole milk

% moisture	Reducing capacity (as mgms. cysteine—HCl/gm.)					
	Weeks storage					
	Initial	4	8	12	16	20
	Lot 384					
1.89	4.00	3.70	3.50	3.24	3.34	4.23
2.29	3.98	3.60	3.56	3.63	3.44	4.14
3.69	3.96	3.78	3.70	3.95	3.65	3.96
4.39	3.90	4.05	5.61	6.22	8.55	9.47
	Lot K33					
1.75	1.61	1.96	1.88	1.74	1.86	
2.82	1.75	1.97	1.86	1.91	2.08	
3.81	1.58	2.05	2.07	2.28	2.38	
4.33	1.61	2.28	2.16	2.86	2.72	

commercial powder (lot 384) was somewhat greater than twice that of the powder made in the experimental drier (lot K33). This difference may be due in part to a greater reducing capacity of the fluid milk, but probably is due primarily to differences in heat treatment during processing [see Crowe *et al.* (6)]. There was a slight decrease in the acid ferricyanide reducing capacity of the samples stored at 20° C., and except for the powder of the highest moisture content (4.39%), there was a slight decrease in the acid ferricyanide reducing capacity of the commercial powder up to 16 weeks storage at 37° C. The values at 20 weeks were about equivalent to those for the fresh powder. The acid ferricyanide reducing capacity of the samples highest in moisture increased materially during storage. The reducing effect of the low-moisture experimental powder samples was virtually unchanged during storage for 16 weeks at 37° C., but there was a definite, although minor, increase in the higher moisture samples.

TABLE 2

Effect of storage at 20° C. on the acid ferricyanide reducing capacity of dry whole milk

% moisture	Reducing capacity (as mgms. cysteine—HCl/gm.)					
	Weeks storage					
	Initial	8	16	24	32	
	Lot 384					
1.90	4.00	3.46	2.97	3.32	3.11	
2.32	3.98	3.49	2.94	3.32	3.05	
3.81	3.96	3.51	3.28	3.19	3.10	
4.46	3.90	3.49	3.30	3.30	3.11	
	Lot K33					
1.75	1.61	1.61	1.56	1.49	1.45	
2.82	1.75	1.61	1.64	1.47	1.47	
3.81	1.58	1.72	1.55	1.49	1.49	
4.41	1.61	1.71	1.77	1.53	1.56	

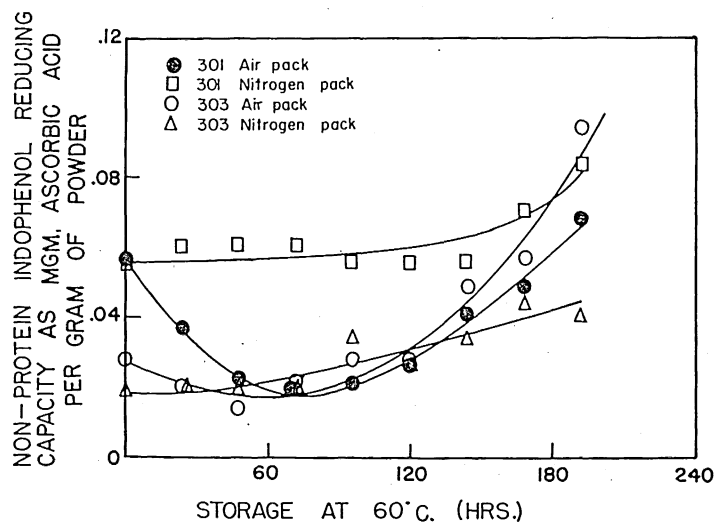


FIG. 5. Change in non-protein indophenol reducing capacity of dry whole milk stored at 60° C.

These data demonstrate the marked effect of temperature on the rate of production of acid ferrieyanide reducing substances. At 20° C. the rate is so slow as to be negligible. At 37° C. the reaction is of minor importance except in powders of higher moisture content.

Ascorbic acid changes. The apparent ascorbic acid content of the samples

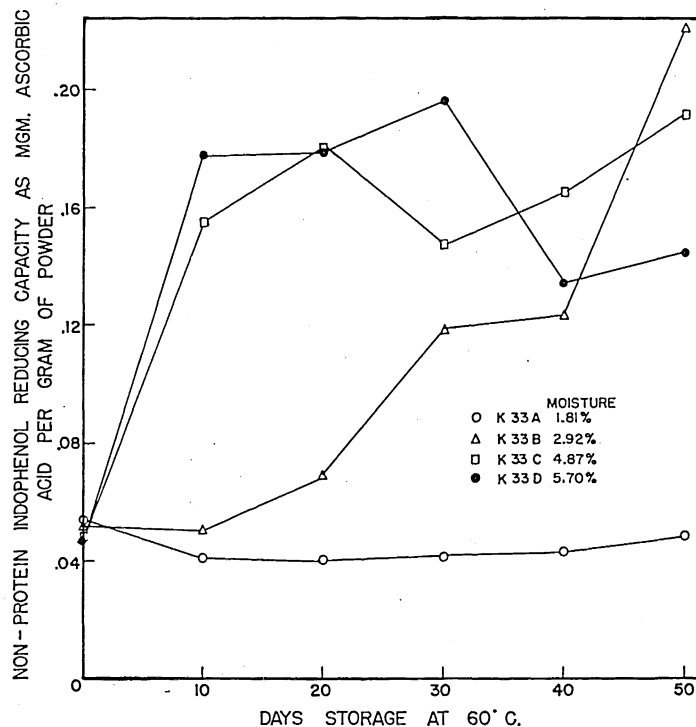


FIG. 6. Change in non-protein indophenol reducing capacity of dry whole milk stored at 60° C.

was determined on the protein-free filtrates titrating with 2,6-dichloro-benzenoneindophenol. The protein-free filtrates were secured by precipitation either with tungstic acid in the manner described by Crowe *et al.* (6), or with a mixture of trichloroacetic and metaphosphoric acids according to the procedure outlined by Doan and Josephson (7). In the presence of oxygen, the ascorbic acid is gradually oxidized in dry whole milk. As shown in figures 5 and 6, substances which react with indophenol are produced particularly in powders of higher moisture.

Fluorescence changes. Fluorescence was determined by the method of Jenness and Coulter (13). Since the oxygen content appeared to be without effect on fluorescence changes during storage, the results at each moisture level have been averaged without regard to oxygen level.

No change in fluorescence of either extract I or II resulted from storage at 20° C., but sample 384 showed a marked increase in fluorescence of extract III which was not duplicated by sample K33. The greater susceptibility of sample 384 to development of fluorescence was even more apparent on storage at 37° C. Sample K33 exhibited scarcely any increase in fluorescence of extract I and only a small increase in extract III during storage for 16 weeks at this temperature. In sample 384, on the other hand, marked increases in fluorescence of both of these extracts occurred. The fluorescence of extract II was unaffected in either sample. Figure 7 shows the changes in fluorescence of sample K33 during storage for 50 days at 60° C. Under this more drastic condition, the fluorescence of extract I increased sharply if the moisture content was sufficiently high, and some increase was noted in fluorescence of extract II. The effect on extract III is interesting in that at moisture contents in excess of about 4 per cent, the initial sharp rise was followed by a pronounced decrease in fluorescence, due either to destruction or insolubilization of the fluorescing materials.

In general, then, it appears that temperature and moisture level determine whether and to what extent fluorescing materials are produced during storage. A considerable difference between powders in susceptibility to production of these materials is also evident.

Production of carbon dioxide. The head-space gas of the cans was analyzed for carbon dioxide and oxygen with a Fisher Precision Gas Analyzer equipped with a Continental Can Company sampling device. All readings were computed to standard temperature and pressure in the manner described by Coulter and Jenness (5).

Carbon dioxide was produced at rates varying with the temperature and the moisture content. Typical data for the powder held at 60° C. are shown in figure 8. Since the rate of production of CO₂ appears to be essentially a straight line function of time over the period studied, the regression line for each powder was computed. The log slopes of the regression lines for the samples held at 60 and 37° C. are shown in figures 9 and 10, plotted against the moisture content. The 60° C. data include those for lot K33 and those for the samples in

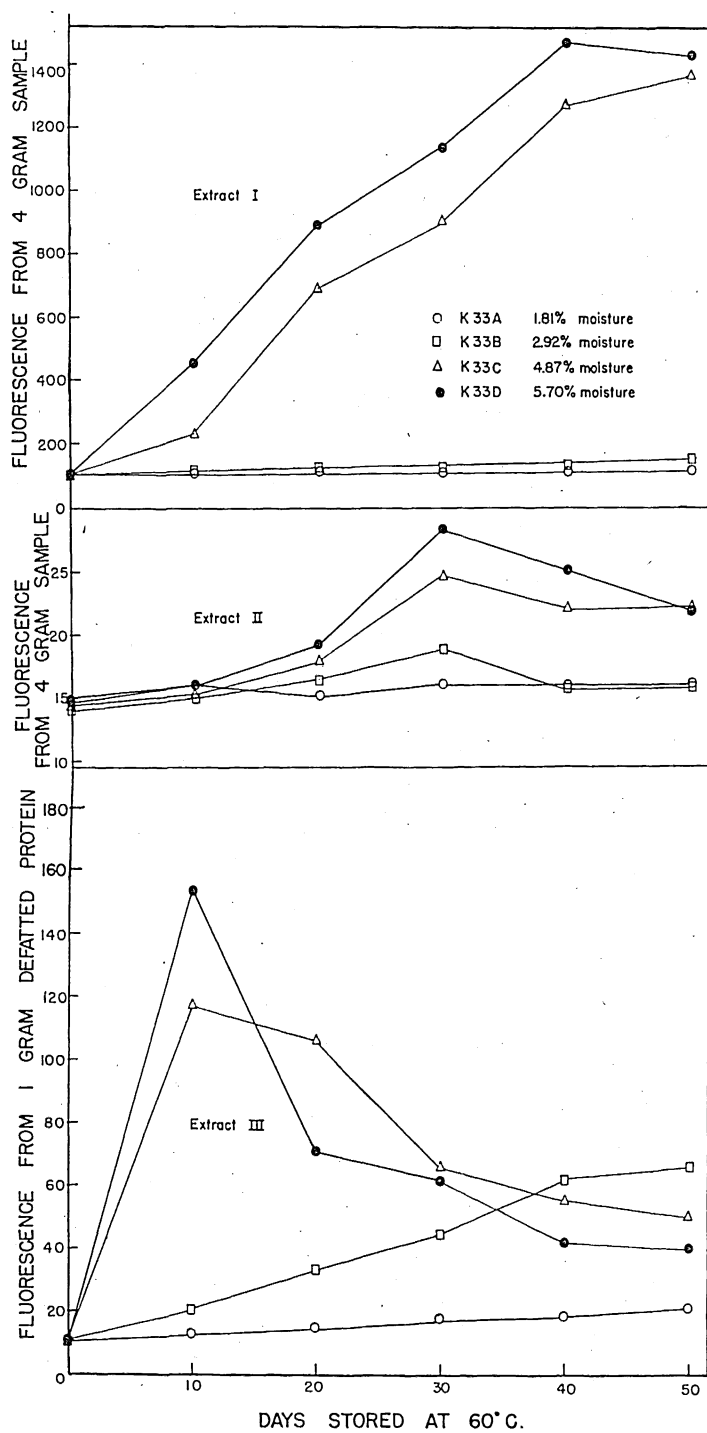


FIG. 7. Change in extractable fluorescence of dry whole milk stored at 60° C.

the initial 9 lots all of which were held for only 10 days. Although the data show considerable scatter, it is evident that production of CO_2 at any given temperature is an exponential function of the moisture content. The effect of oxygen level on the rate of production of CO_2 is not entirely clear. As shown in figure 9, the rate of production of CO_2 in the air-packed samples appears to be slightly higher than in the gas-packed samples. However this difference is far from uniform, and the effect of the oxygen level, if it is a factor, is minor in comparison to that of the moisture content. Production of CO_2 in the samples held at 20°C . was so slow that consistent rate data were not obtained.

Since the powder absorbs some of the carbon dioxide, Coulter and Jenness (5) and Pearce (19), the total carbon dioxide produced cannot be computed from the volume and analysis of the gas.

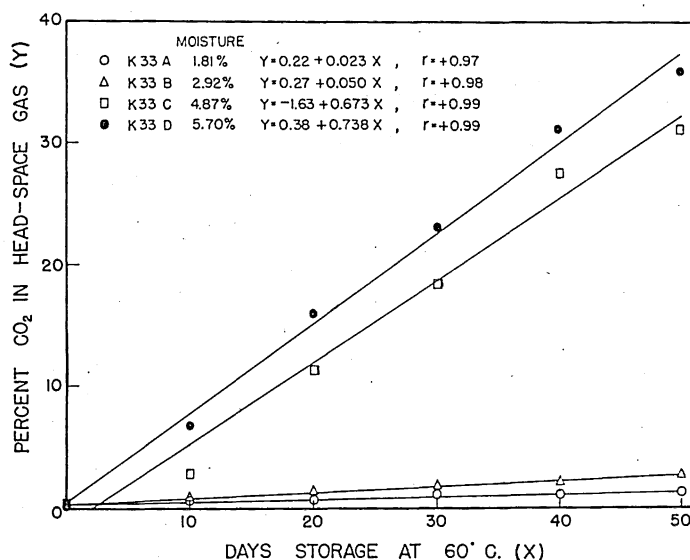


FIG. 8. Change in per cent CO_2 in head-space gas of dry whole milk stored at 60°C .

Oxygen utilization. Only a limited amount of data were secured which were adequate to establish rates of oxygen utilization. These were for the initial nine lots which were held at 60°C . for 10 days. The regression lines for oxygen utilization for each of the air-packed samples were computed and log slopes of the regression lines are shown plotted against the per cent moisture in figure 11. These data indicate that the rate of oxygen utilization increases logarithmically with increase in the moisture content. With these samples there was a very high correlation (+0.92) between the rate of oxygen utilization and carbon dioxide production. Since the rate of carbon dioxide production was only slightly greater in the air-packed than in the nitrogen packed samples, direct oxidation involving the free-space oxygen can play only a minor role in carbon dioxide production.

Production of water. The moisture content of the samples was determined at each examination period using the American Dry Milk Institute toluene dis-

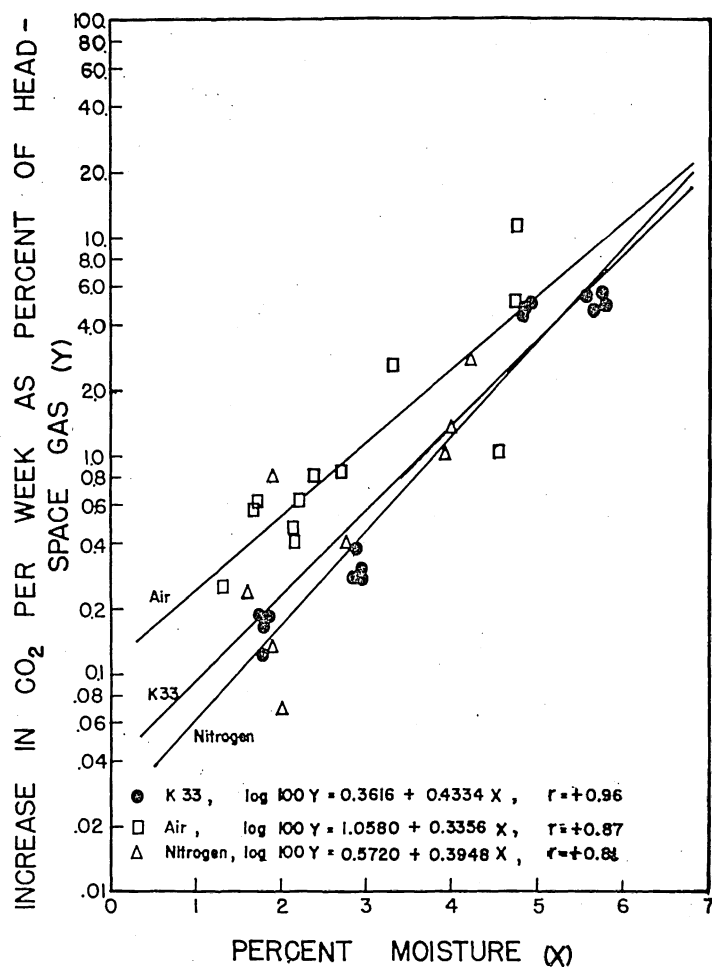


FIG. 9. Relationship of moisture content to rate of increase in CO₂ in head-space gas of dry whole milk stored at 60° C. in air and nitrogen. Sample K33 was stored at oxygen levels ranging from about 1 to 6 per cent.

tillation method. Since the oxygen content appears to be without effect on changes in the moisture content during storage, the results at each moisture

TABLE 3
Effect of storage at 60° C. on the moisture content of dry whole milk

Days of storage					
0	10	20	30	40	50
% moisture					
1.90	1.90	1.60	1.80	1.76	1.90
2.88	3.00	2.95	2.95	2.89	3.02
3.81	4.13	4.95	5.13	5.25	5.95
4.49	5.15	5.94	5.78	6.24	6.53

level have been averaged without regard to oxygen level. The data for the samples stored at 60° C. are shown in table 3. There was a definite increase in the moisture content of the samples having initial moisture levels of 3.81 and 4.49 per cent but not in lower moisture content samples. There was no change in the moisture content of any of the samples stored at 37 and 20° C.

Change in protein solubility. The soluble nitrogen of the samples was de-

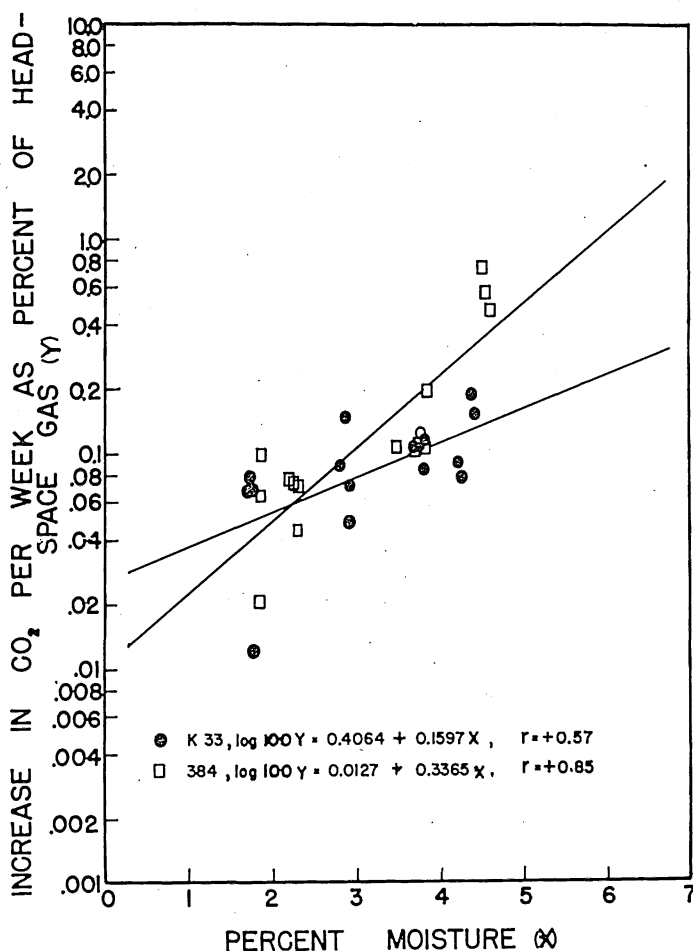


FIG. 10. Relationship of moisture content to rate of increase in CO₂ in head-space gas of dry whole milk stored at 37° C.

terminated on an aliquot taken from the center portion of the centrifuge tube following treatment of the milk according to the American Dry Milk Institute method for solubility index. This method was not entirely satisfactory for those samples which had become brown, since the treatment did not effect a sharp separation of the insoluble material. Filtration of the brown samples was found more satisfactory. The results for the sample stored at 60° C. are summarized in table 4. Since the oxygen level was without effect on protein

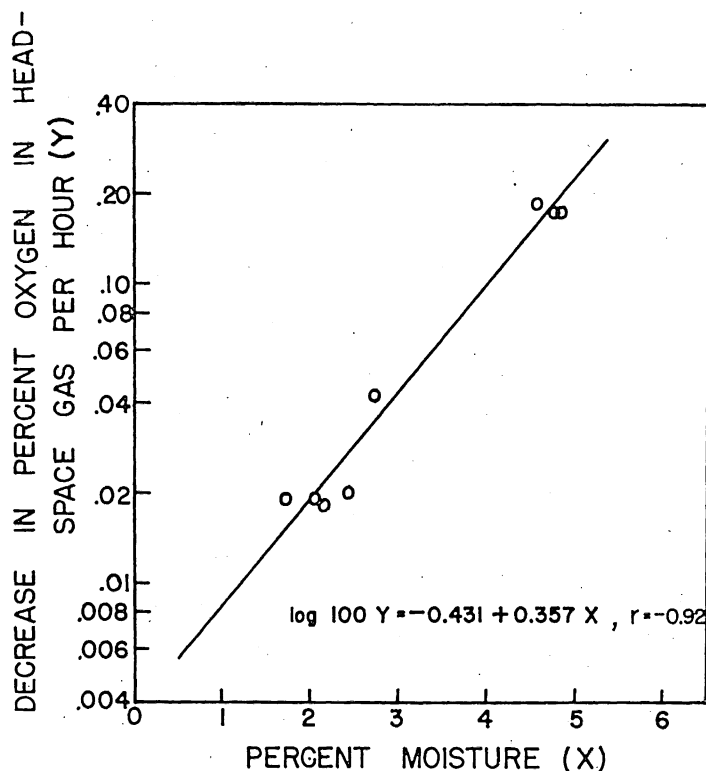


FIG. 11. Relationship of moisture content to rate of decrease in oxygen content of head-space gas of dry whole milk stored at 37° C.

insolubilization, the values reported are the averages at each moisture level. At the end of 50 days storage, the soluble nitrogen in the samples containing 4.49 per cent moisture had been reduced to 6 per cent of the original value. This means that virtually all of the protein was rendered insoluble by the experimental conditions. The solubility of the nitrogenous substances was not

TABLE 4

The effect of moisture level and storage at 60° C. on the lactose, soluble nitrogen and pH of dry whole milk

Storage period in days	% lactose ^a				Soluble N (Mg. per 100 ml. ^a)				pH			
					Initial % Moisture							
	1.90	2.87	3.81	4.49	1.90	2.87	3.81	4.49	1.90	2.87	3.81	4.49
0	36.30	36.65	36.05	36.03	503	502	505	508	6.47	6.49	6.49	6.41
10	36.05	34.30	33.67	33.27	506	495	90.2 ^b	70.3	6.49	6.46	6.17	5.87
20	35.30	33.30	33.17	32.40	504	482	52.7	39.7	6.47	6.32	5.63	5.64
30	35.20	33.23	32.57	31.57	502	458	38.6	33.0	6.45	6.48	5.43	5.38
40	34.85	33.06	32.20	31.37	489	452	36.4	30.3	6.42	6.40	5.32	4.97
50	35.75	33.55	32.56	31.57	495	468	29.5	30.4	6.48	6.32	5.10	4.99

^a Moisture-free basis.

^b The average of two filtered samples, the average of two centrifuged samples being 394.

affected by storage at 60° C. at the 1.90 per cent moisture level and only slightly affected at 2.87 per cent moisture. The solubility of the protein was unchanged in all of the samples stored at 37 and 20° C.

Change in lactose content. The lactose was determined by a modification of the chloramine-T method of Hinton and Macara (10), which is based on the stoichiometric oxidation of the aldehyde group of the sugar. Zinc hydroxide was used for the deproteinization of the milk as suggested by McDowell (17).

The per cent of lactose in the samples stored at 60° C. is shown in table 4. Since the oxygen level did not affect the lactose content, the values shown are the averages at each moisture level. Storage of dry whole milk with an initial moisture content of 4.49 per cent at 60° C. for 40 days resulted in a maximum loss of 15 per cent of the lactose as measured by this method. The lactose decreases most rapidly during the first 10 or 15 days of storage under these conditions. The tendency for an increase in the lactose content at 50 days over that at 40 days may be due to a reduction of the chloramine-T reagent by the increased concentration of competitive reducing systems in the browned milk. The decrease in reducing sugar at the 1.90 per cent moisture level was only 4 per cent.

The lactose content of the samples stored at 37 and 20° C. was unchanged.

Changes in pH. The pH of the reconstituted samples was determined using a Leeds and Northrup glass-electrode pH meter. The pH values for the samples stored at 60° C. are shown in table 4. The data reported are average values at each moisture content, as the oxygen level had no effect on the pH of the reconstituted milk. There was a marked decrease in the pH of the samples having an initial moisture content of 4.49 per cent; a lesser decrease at the 3.80 and 2.87 per cent moisture levels; but none at 1.90 per cent. The pH of the samples stored at 37 and 20° C. for 20 and 32 weeks, respectively, was unchanged.

DISCUSSION

Minimal oxygen levels are considered desirable for the storage of dry whole milk to prevent oxidative changes. Minimal moisture levels have not been considered optimal, because some moisture is necessary for the effectiveness of certain antioxidants. In adequately gas-packed powder, however, oxidation cannot occur.

Numerous changes take place in dry whole milk during storage which are accelerated by increase in the moisture level. These include development of stale, or, at higher moisture levels, a burnt feathers flavor, production of acid ferri-cyanide reducing substances, production of carbon dioxide and utilization of oxygen. The rate of change of each of these, at least during the initial stages, has been shown to increase logarithmically with increase in moisture content. Other changes also increasing in rate with increase in moisture content but for which adequate rate data were not secured are: production of indophenol reducing substances, production of water, production of extractable fluorescent materials, browning, loss of lactose, increase in acidity and loss of

protein solubility. Thus, in adequately gas-packed dry whole milk, a minimal moisture content appears to be desirable; however, the rates of change in every instance are very slow in powders containing 2 per cent or less moisture.

Barker (1) in 1933 observed a logarithmic increase in the rate of heat denaturation of egg albumin with increase in the relative vapor pressure. He explained his observations on the basis that the relative humidity affected the freedom of the water molecules to move between and among the relatively immobile protein molecules and aggregates. He considered this interpretation pertinent regardless of whether the water was a reactant or merely a medium in which the reaction occurred. A similar relationship appears to hold for the reactions involved here.

Although vapor pressure determinations were not made on the samples involved in these trials, determinations on other samples covering the same range in moisture content showed that there is an essentially linear relationship between the per cent moisture and the relative vapor pressure.

All of the reactions mentioned are accelerated greatly by increase in temperature. The magnitudes of the various changes are unimportant except in samples which are higher in moisture than is considered desirable in commercial practice. Therefore, none of the objectively measurable changes can be considered as an effective index of deterioration in commercial dry whole milk.

SUMMARY AND CONCLUSIONS

The following non-lipid changes occur in dry whole milk during storage: development of a stale or burnt feathers flavor, production of acid ferricyanide and indophenol reducing substances, production of carbon dioxide, utilization of oxygen, production of water, production of extractable fluorescent materials, browning, loss of lactose, increase in acidity and loss in protein solubility. All of these changes increase in rate with increase in moisture content and temperature but appear to be relatively unaffected by oxygen.

The rate of change in those variables for which adequate data were obtained increased logarithmically with increase in the vapor pressure of the water in the system, at least during the initial stages.

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Because sufficient funds were unavailable at Ohio State University for research in dairy technology, Professor Stoltz, as an honorary member of the Board of Trustees of the Ohio Dairy Products Association in an advisory capacity, suggested and worked diligently toward the promotion of a research fund by the dairy industry in the State. This fund, known as the Ohio Dairy Products Research Fund, became a reality and today amounts to considerably more than \$100,000, the interest on which at 6 per cent is used for research in dairy technology at The Ohio State University. It has since been suggested that the name of this fund be changed to the Robert B. Stoltz Memorial Research Fund.

Professor Stoltz was listed in *Who's Who in America*, *Who's Who in American Education*, and in *American Men of Science*. He was a member of the Ohio Post-War Program Commission, and of the Columbus Rotary Club.

Professor Stoltz was very active in Masonry, and if ever a man was a true Mason and lived up to the Masonic Creed, it was Bob Stoltz. He was a 33rd degree Mason, and was elected this year as Deputy General Grand Master of the General Grand Council, R. & S. M., of the United States; had served as Grand Master of the Grand Council, R. & S. M. of Ohio; was Past Master of University Lodge and for 14 years its secretary; a member of York Chapter, R. A. M.; York Council, R. & S. M.; Columbus Commandery, Scottish Rite; Aladdin Temple; and Red Cross Constantine.

He was a member of Acacia Fraternity, and Delta Theta Sigma and Gamma Sigma Delta honorary fraternities.

Professor Stoltz was a native of Bradford, Ohio, where he was born March 6, 1890. Surviving are his widow, Mrs. Marie Cassel Stoltz, a son, Philip, three daughters, Mrs. Bonnie Marie Downes, Mrs. Susan Ann George, and Mrs. Roberta Mary Miles.

"Patience, kindness, generosity, humility, courtesy, unselfishness, good temper, guilelessness, sincerity—these make up the supreme gift, the stature of the perfect man." If that is so, then Bob Stoltz was a perfect man because he possessed all of them to a very marked degree.

L. H. BURGWALD

THE INFLUENCE OF WATER LEVEL AND TEMPERATURE OF STORAGE ON CAROTENE PRESERVATION IN DEHYDRATED ALFALFA, CEREAL GRASSES AND MIXED FEEDS¹

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In earlier papers (2, 3), it was pointed out that the carotene of dehydrated alfalfa or dehydrated cereal grasses could be preserved completely if the water content was adjusted to 12 to 20 per cent level and the material stored at 22 to 25° C. in airtight containers. It was postulated that this preservation was related to the speed of reaction of respiratory enzymes which in turn was related to the moisture content of the material.

EXPERIMENTAL

Further studies now have been made with lower water levels in these dehydrated materials stored for 3 months at 22 to 25° C. and 33 to 36° C., respectively. The water levels ranged from 0.9 to 15 per cent with graded increments generally of 2.5 per cent. The materials used were: (a) A dehydrated commercial alfalfa prepared in October, 1947, with an initial carotene content of approximately 350 γ per g. and a water content of 3.6 per cent. (b) An alfalfa cut from a University field in September, 1946, and dried in the laboratory at 50° C. This product was dried further in a vacuum oven for 24 hours at 50° C. prior to starting the experiment. The material, when put up for experimental observation, had a carotene content of approximately 150 γ per g. It consisted of both stem and leaf. (c) A dehydrated alfalfa² which was dried for 2.5 hours at 95° C. before being used in these water level experiments. It contained 154 γ of carotene per g. (d) A dehydrated cereal grass² which contained approximately 160 γ of carotene per g. and was dried for 2.5 hours at 95° C. before being used in the experiments.

All of these materials were adjusted to different water levels and placed in ice cream cartons holding about 250 g. The control was unwaxed, and the remaining cartons were dipped several times in Flexowax to insure complete exclusion of oxygen. They then were stored at 22 to 25° C., and duplicate sets stored at 33 to 36° C. for 3 months. At the end of that time, the cartons were opened, sampled for carotene determination and observations on color and aroma made. The data giving the results are found in tables 1 through 4.

Since it was possible to preserve the carotene in these dehydrated materials

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² Supplied by the Cerophyl Laboratories, Inc., Kansas City, Mo.

TABLE 1

Effect of water level and temperature on carotene, color and pressure of dehydrated alfalfa a.
(Carotene data on water free basis)

Treatment	Color	Gas pressure	% water	Carotene content		
				Initial (γ /g.)	3 months (γ /g.)	% loss
Stored at 22 to 25° C.						
No seal	Green	None	5.2	338	118	67.0
Sealed	Green	None	0.9	338	223	34.0
Sealed	Green	None	2.5	338	248	26.0
Sealed	Green	None	5.0	356	338	5.3
Sealed	Green	None	7.5	356	344	3.6
Sealed	Green	None	10.0	356	362	0.0
Sealed	Slight olive green	None	12.5	356	371	0.0
Sealed	Olive green	None	15.0	356	376	0.0
Stored at 33 to 36° C.						
No seal	Green	None	3.6	338	66	81.5
Sealed	Green	None	0.9	338	230	32.0
Sealed	Green	None	2.5	338	259	23.0
Sealed	Green	None	5.0	356	329	8.0
Sealed	Green	None	7.5	356	348	2.5
Sealed	Olive green	Positive	10.0	356	350	2.0
Sealed	Brown	Positive	12.5	356	363	0.0
Sealed	Brown	Positive	15.0	356	356	0.0

by the procedure outlined, it seemed important to study the losses of carotene in a mixed feed such as is often used in dairy, poultry and hog rations. The ration fed consisted of: soybean meal, 20 per cent; wheat middlings, 20 per cent; wheat bran, 10 per cent; white corn, 21 per cent; oats, 10 per cent; alfalfa

TABLE 2

Effect of water level and temperature on carotene, color and pressure of air dried alfalfa b.
(Carotene data on water free basis)

Treatment	Color	Gas pressure	% water	Carotene content		
				Initial (γ /g.)	3 months (γ /g.)	% loss
Stored at 22 to 25° C.						
No seal	Green	None	6.9	164	122	25.6
Sealed	Green	None	3.4	157.6	129	18.2
Sealed	Green	None	5.0	157.6	136	13.7
Sealed	Green	None	7.5	157.6	144	8.4
Sealed	Green	None	10.0	157.6	146	7.0
Sealed	Slight olive green	None	12.5	157.6	159	0.0
Sealed	Olive green	None	15.0	164	162	1.2
Stored at 33 to 36° C.						
No seal	Green	None	5.2	164	70	57.0
Sealed	Green	None	3.4	157.6	115	27.0
Sealed	Green	None	5.0	157.6	120	24.0
Sealed	Green	None	7.5	157.6	131	17.0
Sealed	Slight olive green	None	10.0	157.6	140	11.0
Sealed	Slight olive green	Positive	12.5	157.6	136	14.0
Sealed	Olive green	Positive	15.0	164	147	11.0

TABLE 3

*Effect of water level and temperature on carotene, color and pressure of dehydrated alfalfa c.
(Carotene data on moisture free basis)*

Treatment	Color	Gas pressure	% water	Carotene content		
				Initial (γ/g.)	3 months (γ/g.)	% loss.
Stored at 22 to 25° C.						
No seal	Green	None	4.4	154	95	38.0
Sealed	Green	None	3.7	154	111	28.0
Sealed	Green	None	5.0	154	117	24.0
Sealed	Green	None	7.5	154	138	10.4
Sealed	Green	None	10.0	154	139	9.7
Sealed	Slight olive green	None	12.5	154	149	3.2
Sealed	Slight olive green	None	15.0	154	159	0.0
Stored at 33 to 36° C.						
No seal	Green	None	3.1	154	68	55.0
Sealed	Green	None	3.7	154	104	32.0
Sealed	Green	None	5.0	154	116	24.0
Sealed	Green	None	7.5	154	132	14.0
Sealed	Slight olive green	None	10.0	154	144	6.5
Sealed	Olive green	None	12.5	154	154	0.0
Sealed	Olive green	Positive	15.0	154	159	0.0

meal, 15 per cent; CaCO_3 , 2 per cent; $\text{Ca}_3(\text{PO}_4)_2$, 1 per cent; and iodized salt, 1 per cent. The only major source of carotene in this feed was the 15 per cent alfalfa meal. The feed was stored at 33 to 36° C. for 3 months with water levels

TABLE 4

Effect of water level and temperature on carotene, color and pressure of Dehydrated cercal grass d. (Carotene data on water free basis)

Treatment	Color	Gas pressure	% water	Carotene content		
				Initial (γ/g.)	3 months (γ/g.)	% loss
Stored at 22 to 25° C.						
No seal	Green	None	4.4	166	110	34.0
Sealed	Green	None	1.6	166	115	31.0
Sealed	Green	None	2.5	166	123	26.0
Sealed	Green	None	5.0	166	133	20.0
Sealed	Green	None	7.5	166	146	12.0
Sealed	Green	None	10.0	166	155	6.3
Sealed	Faint olive green	None	12.5	166	161	3.0
Sealed	Olive green	None	15.0	166	174	0.0
Stored at 33 to 36° C.						
No seal	Green	None	3.0	166	74	55.0
Sealed	Green	None	1.6	166	104	37.0
Sealed	Green	None	2.5	166	112	33.0
Sealed	Green	None	5.0	166	136	18.0
Sealed	Faint olive green	None	7.5	166	150	9.7
Sealed	Olive green	None	10.0	166	164	1.2
Sealed	Olive green	None	12.5	166	164	1.2
Sealed	Brown	None	15.0	166	172	0.0

ranging from 2.3 to 15 per cent under sealed and unsealed conditions. The initial moisture was reduced by drying for 2.5 hours at 95° C. A companion series also was set up but with the addition of certain trace elements now commonly used in such mixed feeds. This series which contained the trace elements was dried initially for 32 hours at 50° C. in a vacuum oven. The trace elements used were 0.02 per cent $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, 0.02 per cent $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.25 per cent $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ and 0.02 per cent $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$.

At the end of 3 months, these cartons were opened, carotene determinations made, as well as observations on color, flavor, aroma, rancidity and other characteristics. The initial carotene content of this feed was 39.6 γ per g. It is assumed that practically all of the carotene was in the 15 per cent of alfalfa meal.

The determination of carotene in the mixed feed by the Wilkes method (4) gave high results. This method seemed entirely satisfactory where only alfalfa or a cereal grass was involved, but when applied to the mixed feed with 15 per cent of alfalfa, the results often were 50 per cent too high. This assumes that there was complete carotene conservation with 12.5 to 15 per cent of water when sealed. Apparently, certain interfering pigments which developed during storage were registering as carotene by the Wilkes method.

The Wilkes method was abandoned for the mixed feed analysis, and the general procedures for chromatographic analysis of carotene which are given in *Methods of Vitamin Assay* (1) were incorporated in the determination. The extraction consisted of allowing 1 to 2 g. of sample to stand 16 to 18 hours (in the dark) in 60 ml. of a 2:1 mixture of Skelly B and acetone. After filtering to remove the sample, the extract was evaporated on a steam bath to reduce the total volume to about 50 ml. Saponification was accomplished next by adding 80 ml. of 5 per cent alcoholic KOH to the extract and allowing the solution to stand in the dark for at least 15 minutes.

The Skelly B phase was obtained by the addition of 40 ml. of water. Following re-extraction of the alcoholic phase twice with 25 ml. portions of Skelly B, the combined Skelly extracts were washed five times with distilled water. The extract was evaporated down, and the final traces of moisture were removed under vacuum. Ten to 15 ml. of Skelly B were added immediately to the dried pigments which now were ready to be chromatographed. The adsorbent employed in the column was a 1:1 mixture of MgO (Micon Brand, no. 2641, Westvaco Corp., Newark, Cal.) with Hyflo Super-Cel (Johns Manville). Following adsorption, the pigments were eluted with a 2 per cent solution of dry acetone in Skelly B. The pigment passing through the column was considered as carotene, and the carotene content of the sample was calculated by standard procedures using pure β -carotene as the reference standard.

The data secured in this experiment are shown in table 5. The striking observation made on these samples was the rancid odor and bleached color in the non-waxed carton containing the trace elements and the total absence of these characteristics when the oxygen was excluded by waxing. When the oxygen was excluded, the greenish color and clean pleasant aroma persisted in all the samples, although with 15 per cent of water the color was slightly olive green.

TABLE 5

Effect of water level, temperature, trace elements, on carotene, color, aroma and pressure of mixed feed with 15 per cent alfalfa stored at 33 to 36° C.
(Carotene data on water free basis)

Treatment	Color	Gas pressure	% water	Carotene content		
				Initial (γ/g.)	3 months (γ/g.)	% loss
<i>No trace elements, Fe, Cu, Mn, Co</i>						
No seal	Bleached	None	3.6	39.6	2.7	93.2
Sealed	Green	None	2.3	39.6	13.7	65.4
Sealed	Green	None	5.0	39.6	16.6	58.1
Sealed	Green	None	7.5	39.6	34.4	13.1
Sealed	Green	None	10.0	39.6	40.8	0.0
Sealed	Green	None	12.5	39.6	41.0	0.0
Sealed	Slight olive green	Positive	15.0	39.6	42.6	0.0
<i>Plus trace elements, Fe, Cu, Mn, Co</i>						
No seal	Bleached ^a	None	4.3	39.6	1.1	97.3
Sealed	Green	None	3.2	39.6	3.8	90.5
Sealed	Green	None	5.0	39.6	6.9	82.5
Sealed	Green	None	7.5	39.6	27.0	31.8
Sealed	Green	None	10.0	39.6	41.8	0.0
Sealed	Green	None	12.5	39.6	46.0	0.0
Sealed	Slight olive green	None	15.0	39.6	46.3	0.0

^aRancid aroma; all other samples had a pleasant aroma.

In the series without the trace elements, the unwaxed material was bleached but possessed a clean non-rancid aroma. In the waxed samples, the greenish color and pleasant aroma persisted in all the samples, although with 15 per cent of water the product was slightly olive green with a slight fermentation aroma.

Involved in the development of the process of carotene preservation in the materials investigated is the question of an economical, practical airtight re-

TABLE 6

Record of preservation of the carotene of dehydrated alfalfa in single thicknesses of Saran tubes (200 gauge). Stored at 33 to 36° C. for 2 months.
(Carotene data on water free basis)

Treatment	Color	Gas pressure	% water	Carotene content		
				Initial (γ/g.)	3 months (γ/g.)	% loss
No seal	Green	None	4.2	377	127	67.0
Sealed ^a	Green	None	7.5	377	368	2.3
Sealed	Green	None	7.5	377	364	3.4
Completely waxed	Green	None	7.5	377	366	2.8
Sealed	Green	None	10.0	377	372	1.3
Sealed	Green	None	10.0	377	346	8.0
Completely waxed	Green	None	10.0	377	372	1.3
Sealed	Olive green	None	12.5	377	363	3.7
Completely waxed	Olive green	None	12.5	377	361	3.9

^a Sealed refers to Saran tubes, waxed only at the end tube joint.

ceptacle. Materials such as tin and iron would be available, but prices probably would prohibit their general use. Sheet aluminum has been tried but is liable to have pin holes and was not effective. Fiber cartons allowed air transmission and gave negative results even when lined with asphalt or paraffin paper liners. Waxed cartons were very effective but the process of waxing did not seem practical. Bags made with Kraft paper treated with Melamine resin were not effective. Among the plastics, polyethylene pouches in Kraft paper bags were tried but found ineffective. The oxygen and carbon dioxide transmission rates of cellophane, nylon, parafilm, pliofilm and polyvinyl alcohol were considered too high to warrant a trial.

Saran, a vinyl-vinylidene chloride copolymer manufactured in various thicknesses was investigated. The 200 gauge material was made into tubes about 1 foot long and sealed at the ends and seams with Flexowax. These tubes were filled with dehydrated alfalfa with varying water content (7.5, 10 and 12.5 per cent) and stored at 33 to 36° C. for 2 months. The records with Saran are shown in table 6. The carotene losses were practically zero. While no claim is made that this material is absolutely negative to oxygen and carbon dioxide transmission, yet the rates of transmission must be very low, even where the alfalfa contained 15 per cent of water. Methods of using this material for the production of a bag or a carton suitable for use in the dehydrated alfalfa and dehydrated cereal grass industry are in progress. It also would appear that such an oxygen-impervious bag or receptacle would find large application in the feed and food industry, where exclusion of oxygen from materials surrounded by an inert gas such as carbon dioxide or nitrogen is desirable.

DISCUSSION

The data on the four dehydrated materials show that a definite level of water with exclusion of oxygen can preserve the carotene of these materials. In a previous study (3), the authors demonstrated the effect of moisture upon the rate of respiration in dehydrated materials. A water content of 5 to 7 per cent or lower apparently does not allow a sufficiently rapid rate of oxygen utilization to prevent significant carotene destruction. Dehydrated materials containing 7.5 per cent or more of moisture when sealed showed good carotene preservation; hence, it is concluded that the oxygen tension in such samples is reduced to a low level in a period of a few days. When the water level is above 10 per cent, the partial destruction of chlorophyll generally supervenes. This is especially true when the storage temperature is as high as 33 to 36° C. for 8 months. Shorter periods of storage at such high temperatures may not affect seriously the green color. Since a green color of the product is much prized by the trade, a water level of about 10 per cent under sealed conditions is recommended to achieve a high preservation of the carotene and maintain the green color.

One must expect variation in the behavior of these dehydrated plant materials to the process outlined. Early harvested materials may have a different rate of respiration than those harvested late in the season. A leaf meal would be

expected to behave differently than a meal composed of both leaf and stem. The season's rain fall, the latitude, the type of soil and the method of dehydration all may have their influence on the behavior of these plants under storage. The length of the period between harvesting and storage also may be an important factor. These problems might well be studied.

The results on carotene preservation in a mixed feed under sealed conditions, with or without trace elements, are especially interesting. That the carotene from only 15 per cent of alfalfa in a mixed feed can be preserved when the oxygen is excluded is important information. It is believed that not only does the alfalfa respire and use up the oxygen when there is a proper moisture content, but that other plant materials also will respire and supplement the activity of the alfalfa. However, this point has not been proven definitely, but since the carotene was preserved in a mixed ration containing only 15 per cent alfalfa (principal carotene source), it is logical to conclude that other plant tissues are contributing to the respiration.

The green color was well preserved when the mixed feed was sealed while the unsealed product became distinctly bleached. Further, in the presence of the trace elements, the unsealed material developed a definite rancid odor, a condition that did not develop in the absence of the trace elements under sealed and unsealed conditions. Consequently, it would seem unwise to add these trace elements to a mixed feed that is to be stored for months and where free access to oxygen is allowed. Some other vehicle, probably common salt, should be used for providing additional trace elements when needed by our livestock.

Many trials were made of materials presumed to be airtight. It is imperative that receptacles for carrying out the outlined process for carotene preservation be airtight, that is, made of materials that will retain the carbon dioxide generated within and prevent the transmission of oxygen into the receptacle. Flexowax was effective but probably impractical. There may be other suitable waxes, but this was the only one tried. Among the plastic films, Saran (Dow Chemical Company) possessed a high preservation quality. It has a high tensile strength and should lend itself to the solution of the problem involved in these studies. Other suitable plastic films may be found.

In practice it is correctly assumed that storage of feed materials with a high water content may lead to the growth of molds and even spontaneous combustion. Both conditions are governed by access to oxygen. With a process that excludes oxygen or greatly lowers its tension, common molds cannot grow and combustion cannot start.

SUMMARY

1. The effect of moisture level and temperature on carotene losses in dehydrated alfalfa and cereal grasses was studied under sealed conditions. The moisture levels studied were 2.5 to 15 per cent and the temperatures employed were 22 to 25° C. and 33 to 36° C.

2. In most instances, almost complete carotene preservation resulted with 10 to 15 per cent of water. Preserving both the carotene and the green color

was best accomplished at 7.5 to 10 per cent of water with 10 per cent as the preferred level because of the more optimum carotene preservation with no detrimental color change. At 7.5 per cent of water, the amount of loss was unpredictable and varied from 2.5 to 17 per cent. The losses increased with decreasing water levels below 7.5 per cent and at 2.5 to 5 per cent varied from 5 to 32 per cent.

3. Storage at 22 to 25° C. (room temperature) was more favorable for the preservation of the green color at 10 to 15 per cent of water level than storage at 33 to 36° C. Little difference in color preservation was observed at either temperature with the moisture below 10 per cent. Postitive pressures seldom were observed with 10 per cent moisture or less and storage at 22 to 25° C.

4. Storage under sealed conditions at 33 to 36° C. of a mixed feed containing 15 per cent alfalfa as the main source of carotene resulted in complete carotene retention with 10 per cent of moisture. Below 7.5 per cent the losses were large. The feed became bleached in the unwaxed carton but retained a pleasant aroma. In waxed cartons feed at any moisture level remained green and had pleasant aromas.

5. Where the mixed feed contained the added trace elements Fe, Cu, Mn and Co, the contents of the unwaxed carton were bleached and also possessed a rancid or tallowy odor. Under sealed conditions the green color and fine aroma were retained, and at 10 per cent and above of water, the carotene was preserved completely.

6. Investigation of many materials as barriers to oxygen and carbon dioxide transmission finally led to the use of Saran, a plastic film. It was found effective for the preservation of carotene in dehydrated alfalfa, with a proper water level.

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STUDIES ON RUMINAL GAS FORMATION AND ON CONSUMPTION OF ALFALFA PASTURE BY CATTLE

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This paper is concerned with the effect of diet on ruminal gas formation and with studies on the consumption of legume pasture. It was shown earlier (2) that no more gas was formed on a bloat-provoking diet such as green alfalfa tops than on a non-bloat-provoking diet such as alfalfa hay and grain. This observation led to the conclusion that increased gas production in itself could not explain acute bloat. In the same paper, it was shown that the rate of gas production depended upon the amount of feed consumed. Furthermore, both the present authors (2) and Quin (6) have shown by introducing gas into the rumen that much more can be expelled by belching than ever is produced in the rumen. Consequently, the hypothesis that acute bloat is due to a lack of sufficient coarse roughage in the rumen to induce eructation has been suggested (2). With this hypothesis, the rate of gas production is still an important consideration because belching rarely, if ever, is completely inhibited on diets low in coarse roughage. On the basis of this theory, it has been possible to induce and prevent bloat at will (3). Nevertheless, fatal bloat is not always produced on all succulent fields, and this failure has appeared to be due to a low consumption of alfalfa.

Quin (6) has suggested that bloat depends on both a high sugar content of the legumes at certain times which accelerates gas production, and a high saponin content which results in foaming with a consequent trapping of the gas and which thus prevents eructation. In support of the importance of the first factor, he submits evidence that glucose added to ruminal contents speeds up gas production more than does the addition of starch. The second postulate was based on his observation that ruminal ingesta from animals fed on alfalfa had a greater tendency to foam than ingesta from animals on other feeds, and on the report of Jacobson (4) that alfalfa contains a saponin with strong foam-producing properties. The results reported in the present paper confirm Quin's finding that glucose, under certain conditions, results in a more immediate increase in gas production than does starch, but further work is necessary to establish the view that changes in sugar content play a major role in determining the incidence or severity of bloat. The present authors have stuck a number of bloated cattle and find that foaming is not the cause of many cases of bloat. In one animal near death, the excess gas easily escaped when the animal was stuck with a trocar cannula, and in another severely bloated animal the excess gas was withdrawn by means of a stomach tube without obstruction by foaming. However, foaming may prevent eructation under certain conditions.

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EXPERIMENTAL

Dairy cows, in most instances lactating Jerseys, were used in the present studies. The method used for determining the rate of gas production has been described (2) as also has the method for determining food consumption on pasture (1). The rumen was tapped for gas production by means of a trocar cannula intended for bleeding horses.

Ruminal Gas Production Studies

Comparison of gas production on green alfalfa tops and on green Sudan grass. Although the rate of gas production on non-bloat-provoking diets such as alfalfa hay and grain has been compared to that on green alfalfa tops,

TABLE 1

Comparison of ruminal gas formation following feeding of Sudan and alfalfa tops. The cows were fed ad libitum throughout the 4-hour experimental period

Cow no.	Pounds of Sudan or alfalfa tops consumed ^a	Cubic feet of gas formed:		
		Half-hour before feeding	Half-hour after feeding	First 4 hours after feeding
Sudan tops				
760	69.7	0.08	0.28	4.02
760	50.4	0.26	0.47	5.43
757	7.5	0.32	0.47	2.77
760	63.0	0.34	0.59	6.70
Av.	47.6	0.25	0.45	4.73
Alfalfa tops				
760	26.9	0.06	0.48	4.17
757	2.4	0.34	0.28	3.29
760	19.7	0.30	0.78	6.82
832	12.9	0.34	0.43	3.00
Av.	15.5	0.26	0.49	4.32

^a In addition to the tops, cow 760 received 4 pounds of a concentrate mix the night before the trial. Cows 757 and 832 received 4 pounds of concentrates the night before and the morning of the trial.

grasses have not been compared with legumes. Because bloat rarely occurs on grasses, this comparison seemed desirable.

The alfalfa and Sudan tops were cut and fed in the barn. The cows were pastured on the field from which the tops were to be taken for two days preceding the trial. No hay was fed the night previous or on the morning of the trial, but the regular concentrate allowance was given (4 to 8 lb. per day, depending on milk production). Gas production was determined over a 30-minute control period before feeding the alfalfa or Sudan. The object of the experiment was to determine the amount of gas formed on the two feeds when cows were given free access to the feed over a 4-hour experimental period. The results are shown in table 1. The gas formation during the first 4 hours after the beginning of feeding was approximately the same for the two feeds, but the average consumption of Sudan was three times that of alfalfa. Conse-

quently, it appears that the rate of gas production would be greater with alfalfa than with Sudan if equal quantities were fed.

Cow 757 ate very little during the gas determination period, as she was distressed by the presence of the cannula. Cow 760, on the other hand, evidenced no discomfort upon insertion of the trocar cannula; she continued to eat and ruminate in a normal manner. These individual differences are mentioned to point out that the use of the trocar cannula in gas production studies necessitates some discrimination in the selection of suitable experimental subjects.

It may be noted that there is an increase in gas production during the first 30 minutes after feeding (table 1). The promptness of acceleration of ruminal gas formation following ingestion of feed is an interesting phenomenon. On a given feed, there are some discrepancies between the amount consumed and the volume of gas formed which are difficult to explain. The amount of feed consumed was not measured on the 2 days preceding the test, and it may be that variations in the volume of ingesta present in the rumen at the beginning of the trial may provide an explanation.

TABLE 2

Comparative effects of glucose and starch on ruminal gas formation in cows given free access to green alfalfa tops for 4 hours preceding the experimental period. Two kg. of starch or glucose in 6 liters of H₂O were administered through a cannula directly into the rumen

Cow no.	Drench	Cubic feet of gas formed:		
		Hour before drench	Hour after drench	Second hour after drench
760	glucose	1.50	1.56	1.69
832	glucose	0.73	0.85	0.76
	Av.	1.12	1.21	1.23
760	starch	1.14	1.14	1.53
760	starch	1.45	1.39	1.34
	Av.	1.30	1.27	1.45

Comparative effects of glucose and starch on gas formation. In the light of Quin's hypothesis and data cited above, it seemed desirable to obtain more information on the effects of glucose and starch on gas formation. The tests were run under two conditions: in the first, the cows were given free access to green alfalfa tops fed in the barn for 4 hours preceding the test period; in the second, the cows were fed 9 lb. of alfalfa hay and 6 lb. of rolled barley 20 hours before the experimental period. Gas production was determined for 1 hour before the experimental period in the first experiment and for 30 minutes in the second. To introduce the test substance, glucose or starch, a rubber tube with a funnel attached to one end was connected to the side arm of the cannula. During the introduction of the test substance, the tube leading from the cannula to the gas meter was clamped off. The solution of starch or glucose was poured into a funnel elevated 3 or 4 feet above the level of the entrance of the cannula into the rumen, the fluid flowing into the rumen by gravity. Five to ten minutes were needed in introducing the solution.

In table 2 is shown the effect of administering starch or glucose to cows fed alfalfa tops for 4 hours preceding the test period. No significant change in the rate of gas formation resulted with either glucose or starch.

The results obtained when the cows were fed 20 hours before the experimental period are given in table 3. The amount of glucose or starch administered was reduced from 2 kg., as in the previous experiment, to 1 kg., because the higher dose of glucose on a partially empty rumen had an adverse effect on the cow, resulting in diarrhea and loss of appetite. Under this regime, 1 kg. of glucose in 3 liters of water increased gas production regularly within the first half hour after its introduction. The response to an equal amount of

TABLE 3

Comparative effects of glucose and starch on ruminal gas formation in cows fed 20 hours before the experimental period. One kg. of starch or glucose in 3 l. of H₂O was administered through a cannula directly into the rumen

Date of trial	Cubic feet of ruminal gas formed:					
	Hour before drench ^a	1st hour after drench	2nd hour after drench	3rd hour after drench	4th hour after drench	Total after drench
1 kg. glucose administered						
Mar. 11	0.33	0.67	0.68	0.51	0.53	2.39
Mar. 23	0.31	1.11	1.11	0.78	0.76	3.77
Mar. 30	0.42	1.09	0.85	0.85	0.52	3.31
Apr. 13	0.44	1.00	0.96	0.83	0.45	3.24
Av.	0.38	0.97	0.90	0.74	0.57	3.18
1 kg. starch administered						
Mar. 18	0.56	0.80	0.94	0.84	0.56	3.13
Mar. 25	0.48	0.45	0.62	0.67	0.56	2.29
Apr. 6	0.48	0.43	0.76	0.98	0.81	2.98
Av.	0.51	0.56	0.77	0.83	0.64	2.80

^a Gas was determined for only 0.5 hr. before drenching. The figure obtained was multiplied by 2 to facilitate comparison of gas production before and after drenching.

starch was not marked until the second hour after drenching, but during the third and fourth hours more gas was liberated than with glucose. The tests on starch and glucose were run for 30 minutes longer than is shown in table 3. During this last half hour, there was an average production of 0.22 cubic feet of gas with glucose and 0.32 cubic feet with starch. Thus the effect of starch is more prolonged and the total gas produced is apparently the same as with glucose.

Studies on Consumption of Alfalfa Pasture

For these studies, the cows were weighed in and out of pasture and during the intervening period all excreta were collected and weighed. The pasturing period extended from 8 a.m. to 2:30 p.m. The studies were made between June 14 and October 10. Insensible losses were determined on 2 days and amounted to approximately 3 lb. per hour, but the insensible losses were not taken into account in calculating feed consumption. On a few occasions, the

insensible losses exceeded feed consumption, thus explaining the apparent negative consumption values shown in figures 1 and 2.

One of the objectives of the experiment was to determine if palatability varied in different fields. Further, it was desired to ascertain the influence of maturity on palatability. Decisive answers were not obtained to either question for reasons which will be explained. The results of the study are summarized in figures 1 and 2.

Figure 1 gives the feed consumption on two different fields, 1-C South and Dairy Field 4. Two lactating cows were used in this part of the study; cow

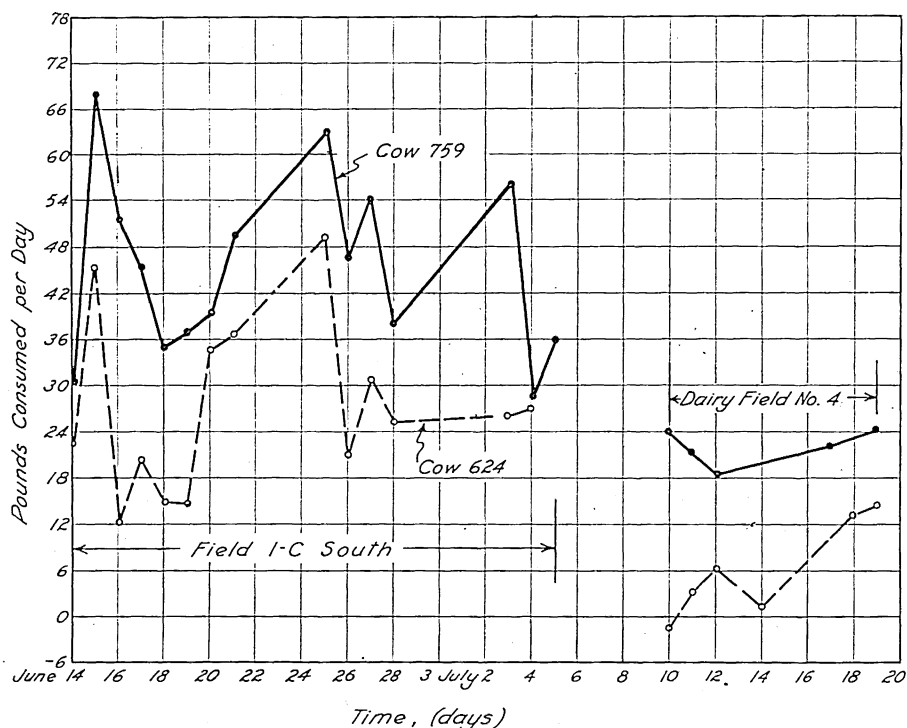


FIG. 1. Consumption of alfalfa on fields 1-C South and Dairy Field 4. The cows, 759 and 624, were on pasture for 6.5 hours daily. In determining feed consumption, insensible losses were not considered.

624 received 5 lb. of a concentrate mix at 3 a.m. and 3 p.m. and cow 759 received 7 lb. night and morning. Cow 759 had been shown to be susceptible to bloat in previous studies, whereas cow 624 never had bloated severely, even under conditions in which the majority of the herd had bloated. No hay was fed. The cows were pastured intermittently on 1-C South from June 14 to July 5. There were 12 acres in this field, and thus the amount consumed by the 2 cows had no appreciable influence on the amount of feed available during this period. Although the stand of alfalfa on 1-C South appeared to be fairly clean on cursory examination, there were some weeds and annual grasses on the irrigation checks. When the cows were first put on the field, the alfalfa was about 1 foot tall and very succulent. Furthermore, the alfalfa was relatively

unpalatable and during the first week the cows ate approximately as much weeds and grasses as alfalfa. One cow did not bloat on this field, and the other, cow 759, bloated slightly on 3 different days. We attribute this relative lack of bloat to the consumption of sufficient weeds and grasses to induce belching. In support of this view is the fact that both cows ruminated more than one would expect when pasturing on succulent alfalfa without access to hay. Cows ruminate very little on fields causing severe bloat. Experience in the next field, Dairy Field 4, with the same cows adds weight to this interpretation. This field had been pastured earlier in the season and was devoid of contaminating

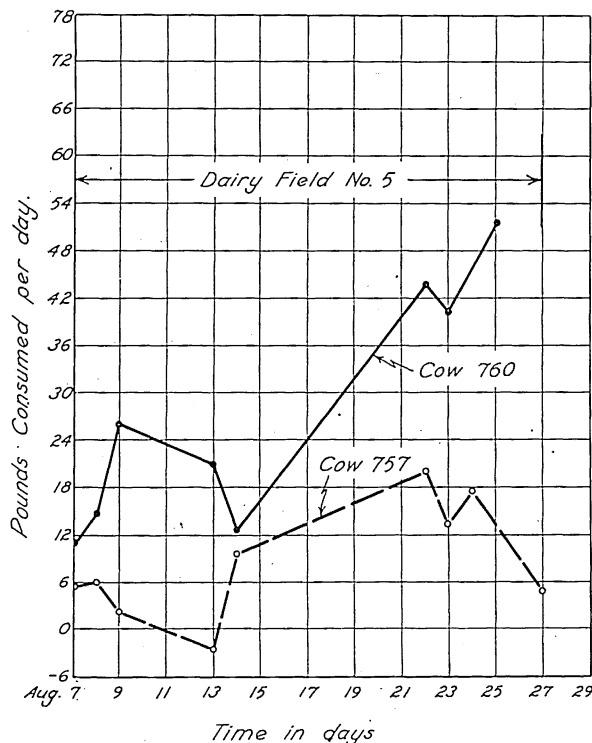


FIG. 2. Consumption of alfalfa on Dairy Field 5. The cows, 760 and 757, were on pasture for 6.5 hours daily. In determining feed consumption, insensible losses were not considered.

weeds and grasses. Cow 624 bloated on 4 of 6 pasturing days on this field, and cow 759 bloated every day—on two occasions the bloat was sufficiently severe to require treatment with turpentine. “Severe bloat” refers to a condition in which there is marked distress, frequent urination and defecation and a ruminal pressure of 45 to 70 mm. Hg (1).

Palatability of the alfalfa on the two fields was considered. Field 1-C South was pastured over a 3-week period during which the alfalfa became progressively more mature. It was in the late bud stage at the termination of the trial. This field was irrigated on June 11. The fact that the cows ate a considerable proportion of weeds, particularly during the first week, presents

some difficulties in interpretation. However, the cows were under constant observation during the pasturing period by the attendants collecting the excreta, and there is little doubt but that the cows ate a greater amount of alfalfa as it became more mature. Furthermore, there is little doubt that the alfalfa in field 1-C South was more palatable than in Dairy Field 4. The results are difficult to evaluate, however, for two reasons: on field 1-C South, the exact consumption of alfalfa is unknown because the cows ate grasses and weeds in addition to alfalfa; secondly, the consumption of alfalfa on Dairy Field 4 was depressed as the result of bloat. In other words, when the cows bloated, they stopped eating.

The results on Dairy Field 5 with cows 760 and 757 are shown in figure 2. Here the evidence seems a little more clear-cut that the alfalfa becomes more palatable as it matures. On August 13 and 14, cow 760 bloated, on the latter date sufficiently severely to require treatment with turpentine. This explains her relatively low consumption on these days. By August 22nd, the alfalfa was in the early bloom stage and was sufficiently coarse to induce frequent rumination. No adequate explanation is available for the relatively low and sporadic feed consumption of cow 757.

DISCUSSION

The results on ruminal gas formation following feeding of green Sudan and alfalfa tops indicate that one might expect a greater amount of gas formed from alfalfa if equal amounts of the two feeds were given. With *ad libitum* feeding, the total gas formed from the two feeds, however, was approximately the same because of the greater consumption of Sudan. Increased gas production on legumes does not in itself provide an adequate explanation of bloat; cows will bloat on amounts of alfalfa comparable to those consumed in these experiments but bloat did not occur when normal animals were given an amount of Sudan producing an equivalent volume of gas. Previous studies (2) have shown that dry legume hay results in as much gas production as green alfalfa. These data give further confirmation, therefore, that it is the inability of animals to eructate the gas on legumes which makes alfalfa and clover dangerous from a standpoint of bloat. Nevertheless, the rapid gas formation on legumes undoubtedly is a contributing factor in bloat.

Quin (5) has compared the rate of gas formation on alfalfa and grass hay. He reports a rapid production of gas on alfalfa hay, a result in accord with our studies (2). On the contrary, he found no gas formed over a 90-minute period in two of three trials with sheep on a basal diet of grass hay. In the light of the data reported herein on a green grass (Sudan), this result needs further confirmation.

When cows were fed 20 hours before the experimental period, glucose caused an earlier increase in gas formation than did starch, but the total gas produced from the two substances over a period of 4.5 hours was about the same. Quin reported that when starch, in the form of maize, was given to sheep maintained on a basal diet of green alfalfa, there was no gas formed over a 90-minute

period. When cows were given a full feed of alfalfa during a 4-hour interval preceding the test period, no difference in gas formation between glucose and starch was observed. Conceivably the sugar content of alfalfa could be a contributing factor in bloat as postulated by Quin, but further studies are necessary to establish the point.

The present studies on palatability of legumes at different stages of maturity were not conclusive but indicated that alfalfa increases in palatability as it matures. Two main difficulties in these studies were encountered: first, cows ate weeds and grasses along with the alfalfa when the fields were contaminated; second, cows on pure alfalfa stands bloated and this in turn depressed feed consumption and made it impossible to obtain a true estimate of palatability. Therefore, it appears that a more desirable procedure would be to cut the alfalfa tops and feed them in the barn. In this way, the weeds and grasses could be avoided. Further, it would appear necessary to supplement the diet with sufficient Sudan grass hay or with green Sudan to obviate bloat.

SUMMARY AND CONCLUSIONS

In an average of four trials, 4.7 cubic feet of gas were produced when cows consumed 47.6 lb. of green Sudan tops fed *ad libitum* over a 4-hour period as compared to 4.3 cubic feet when cows consumed an average of 15.5 lb. of green alfalfa tops over a similar period.

The amount of gas formed following drenching with glucose or starch was determined both by feeding cows 20 hours before the experimental period or feeding them with alfalfa tops *ad libitum* 4 hours preceding drenching. In the former instance, glucose caused a more prompt increase in gas formation, whereas the effect of starch was more prolonged. When cows were fed immediately preceding drenching, on the other hand, no difference between glucose and starch as regards gas formation was discernible.

Studies on the consumption of alfalfa pasture indicate that alfalfa becomes more palatable as it matures up to the early bloom stage, but the results were inconclusive.

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A STUDY OF THE USE OF THE ANTIOXIDANT NORDIHYDROGUAI- ARETIC ACID IN DAIRY PRODUCTS. II. ITS ANTIOXYGENIC PROPERTIES IN UNSWEETENED FROZEN CREAM¹

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The use of antioxidants in retarding the development of oxidized flavor during the storage of frozen cream has been studied by several investigators (2, 3, 4, 5, 6). The work reported herein consists of a study in which nordihydroguaiaretic acid (NDGA) was used to retard the development of oxidized flavor during the storage of unsweetened frozen cream containing 40 per cent milk fat.²

EXPERIMENTAL PROCEDURE

Two grades of cream, one of high and the other of low quality, were used in this study. Standard plate count (1), acidity and pH were the criteria upon which quality was based (table 1).

TABLE 1

The standard plate count, titratable acidity and pH of the raw and pasteurized cream

	High quality		Low quality	
	(a)	(b)	(c)	(d)
<i>Raw cream</i>				
Standard plate count	340,000	32,000	345,000,000	6,000,000
Titratable acidity, as % lactic acid	0.120	0.140	0.155	0.125
pH (25° C.)	6.74	6.63	6.50	6.66
<i>Pasteurized cream</i>				
	150° F.	170° F.	150° F.	170° F.
Standard plate count	900	55	2,000	2,860
Titratable acidity, as % lactic acid	0.125	0.135	0.145	0.140
pH (25° C.)	6.62	6.60	6.50	6.39

The different batches of cream were standardized to contain 40 per cent milk fat. NDGA was added after pasteurization as a 10 per cent solution in glycerol or as a 5 per cent water suspension. The concentrations of NDGA were computed on the basis of the fat content of the cream. When used, copper was added at a concentration of 0.5 p.p.m. in the form of a 0.5 per cent aqueous solution of copper sulfate.

The cream was pasteurized in well-tinned equipment, cooled to 40–45° F., sealed in tinned cans holding 300 ml. and stored at –12 to –20° F. For monthly flavor criticisms, the frozen cream was thawed by holding it 24 hours at 40° F.

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¹ The data contained in this paper are from a thesis submitted by the senior author to the Graduate School of the University of Illinois in partial fulfillment of the requirements for the degree of Master of Science, 1947.

² The legality of adding antioxidant material to dairy products would need to be established before its use could be recommended. The authors' interest in the product studied was mainly scientific, although the practicable possibilities of a study of this nature always must be recognized.

TABLE 2
The antioxidant effect of NDGA added to unsweetened cream stored at sub-zero temperatures

Treatment	Flavor criticisms									
	1 mo.		3 mo.		5 mo.		7 mo.		9 mo.	
	(1) ^a	(2) ^b	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
<i>Pasteurized at 150° F. for 30 min.</i>										
Series A. Low quality cream. No copper added.										
Control	1 ^d	1	1	2	2	4
Control + 0.00125% NDGA
Control + 0.005% NDGA
Series B. Low quality cream. 0.5 p.p.m. copper added.										
Control	2	3	4	5	5 ^{fe}	5 ^f	5 ^f	5 ^f	5 ^f	5 ^f
Control + 0.00125% NDGA	1	1	1	2	4 ^f	4 ^f	5 ^f	5 ^f
Control + 0.005% NDGA	1	1	1	2	4 ^f	4 ^f	5 ^f	5 ^f
Series C. High quality cream. No copper added.										
Control	1	1	1	1	1	...
Control + 0.00125% NDGA
Control + 0.005% NDGA
Series D. High quality cream. 0.5 p.p.m. copper added.										
Control	2	3	3	5	5	5	5	5 ^f	5 ^f	...
Control + 0.00125% NDGA	± ^f	1	1	2	2	2	2	...
Control + 0.005% NDGA	±	1	1	1	1	1	1	...
<i>Pasteurized at 170° F. for 15 min.</i>										
Series E. Low quality cream. No copper added.										
Control	...	±	±	1	1	1	1	1	±	1
Control + 0.00125% NDGA
Control + 0.005% NDGA
Series F. Low quality cream. 0.5 p.p.m. copper added.										
Control	...	±	±	1	1	1	1	1	±	±
Control + 0.00125% NDGA	±	±	±	±	±	±	±
Control + 0.005% NDGA	±	±	±	±	±	±	±
Series G. High quality cream. No copper added.										
Control	1	1	1	1	1
Control + 0.00125% NDGA
Control + 0.005% NDGA
Series H. High quality cream. 0.5 p.p.m. copper added.										
Control	1	1	2	3	4	3	5
Control + 0.00125% NDGA
Control + 0.005% NDGA

^a Flavor judged immediately after taking cream out of storage and thawing it.

^b Flavor judged after thawing cream and then holding it at 40° F. for 1 week.

^c No oxidized flavor present.

^d The numbers 1 to 5 indicate increasing levels of oxidized flavor defect.

^e Fishy flavor.

^f Flavor slightly 'off.' Not typically oxidized.

It then was held at 40° F. for 1 week after which it was judged again for flavor. The judging panel was composed of three or more persons.

RESULTS

The data in table 2 are typical of results obtained with cream which was placed in storage during the months of August and September. There were no significant differences in the antioxygenic effectiveness of the NDGA when added in glycerol solution and when added in a water suspension. Therefore, the data presented in table 2 includes only results from cream treated with NDGA in glycerol solution.

The effect of concentration of NDGA. A concentration of 0.005 per cent NDGA was more effective than one of 0.00125 per cent. This is demonstrated in the results obtained with the high quality cream which contained added copper and which was pasteurized at 150° F. for 30 minutes (table 2, series D). Oxidized flavor had developed at the end of 3 months storage in the control sample. While this off-flavor had developed at the end of 5 months in the cream treated with both 0.00125 per cent and 0.005 per cent NDGA, the intensity of the off-flavor at the end of 7 months was less in the cream containing 0.005 per cent than in the cream containing 0.00125 per cent NDGA.

The effect of pasteurization temperature. Development of oxidized flavor was retarded by pasteurizing the cream at 170° F. for 15 minutes. Oxidized flavor had developed at the end of 7 months storage in the control sample of series A which was pasteurized at 150° F. for 30 minutes, while it did not develop during storage at sub-zero temperatures in the similar cream pasteurized at 170° F. for 15 minutes. Oxidized flavor was present at the end of 1 month in the control sample of series B which was pasteurized at 150° F. for 30 minutes, whereas it did not develop in the similar cream pasteurized at 170° F. for 15 minutes.

There was no oxidized flavor development during storage for 11 months at sub-zero temperatures in the high quality cream which contained no added copper (table 1, series C and G). The off-flavor was present at 3 months in the control sample of series D which was pasteurized at 150° F. for 30 minutes but was not detected until the end of 5 months in the similar cream pasteurized at 170° F. for 15 minutes. While the keeping quality of the cream pasteurized at 170° F. for 15 minutes was superior to that pasteurized at 150° F. for 30 minutes, it had a cooked flavor which persisted throughout the storage period. The keeping quality of the cream which was pasteurized at 150° F. for 30 minutes and which contained NDGA but no added copper was comparable to that of cream pasteurized at 170° F. for 15 minutes.

The effect of quality of the cream. The cream which was pasteurized at 150° F. for 30 minutes developed the oxidized flavor in the control sample containing added copper (series B) at the end of 1 month, but the off-flavor was not detected in the similar high quality cream until the end of 3 months (series D).

There was no oxidized flavor development in any of the low quality cream pasteurized at 170° F. for 15 minutes during storage for 11 months at sub-zero

temperatures. However, the off-flavor was present at the end of 5 months in the control sample of the similar high quality cream containing added copper (series H).

The effect of quality as indicated in this study was variable. The high quality cream which was pasteurized at 150° F. for 30 minutes had a better keeping quality than the similar low quality cream with respect to the oxidized flavor development. The converse of this was true in the cream which was pasteurized at 170° F. for 15 minutes.

The effect of holding the thawed cream at 40° F. for 1 week. Oxidized flavor developed frequently in the control samples which were held at 40° F. for 1 week, although they did not have the off-flavor when they were taken out of storage. This relationship was illustrated in the control sample of series D pasteurized at 150° F. for 30 minutes which had been stored for 1 month at sub-zero temperatures and did not have the oxidized flavor when first removed from the low temperature storage, but developed it after the sample had been held at 40° F. for 1 week. The same observation was made after 5 months in the control samples of series E and after 7 months in the control samples of series H, both of which were pasteurized at 170° F. for 15 minutes.

After storage for 1 week at 40° F., the oxidized flavor usually increased in intensity in the control samples which had that off-flavor when they were first taken out of storage. This is evident in the cream pasteurized at 150° F. for 30 minutes in the control samples of series A after 9 and 11 months, in series B after 1, 3 and 5 months and in series D after 5 months. This trend also was evident after 7, 9 and 11 months in the control samples of cream in series H which had been pasteurized at 170° F. for 15 minutes. However, the intensity of the oxidized flavor did not increase during storage at 40° F. in the samples which contained NDGA.

CONCLUSIONS

1. Concentrations of 0.00125 to 0.005 per cent nordihydroguaiaretic acid were found to retard the development of oxidized flavor in unsweetened frozen cream during storage for 11 months.
2. In the absence of added copper, the keeping quality of the cream which contained nordihydroguaiaretic acid and was pasteurized at 150° F. for 30 minutes was comparable to that pasteurized at 170° F. for 15 minutes but to which the antioxidant had not been added.
3. In this study, the high quality cream pasteurized at 150° F. for 30 minutes had a better keeping quality than the low quality cream similarly pasteurized. The converse of this was true in the cream which was pasteurized at 170° F. for 15 minutes.
4. During storage for 1 week at 40° F., an oxidized flavor developed frequently in the control samples, although these samples did not have the off-flavor when they were taken out of storage at sub-zero temperatures. This did not occur in the cream which contained nordihydroguaiaretic acid.
5. During storage for 1 week at 40° F., the intensity of the oxidized flavor usually increased in the control samples which had the off-flavor when they were

first taken out of storage at sub-zero temperatures. This did not occur in the oxidized samples which contained nordihydroguaiaretic acid.

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THE RELATION BETWEEN THE MONTH OF CALVING AND YEARLY BUTTERFAT PRODUCTION¹

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Approximately 71 per cent of the dairy cows of Oregon are located west of the Cascade Mountains; and about two-thirds of these, or 46 per cent of the total, are found on farms in the ten Willamette Valley counties, a region with an average temperature of 52° F. and a monthly mean range from a high of 65° F. in July to 38° F. in December. The average rainfall of about 42 inches comes mostly during the winter months. Since the climate under these conditions is mild and does not show large seasonal variations in comparison with some other parts of the United States, it became of interest to study the effect of the month of calving on butterfat production.

Differences in yearly milk production between cows freshening in the different months of the year, in various parts of the United States, have been found to exist (1, 2, 5, 7, 8). The season of the year in which the cow freshens also was reported to exert an effect on her butterfat production (1, 4, 8). In Connecticut, Frick *et al.* (2) found that the differences in milk production of cows calving in the different months of the year were highly significant statistically.

PROCEDURE

Data for the present study were obtained from the record books of the dairy herd owned by Oregon State College and from official test records of cows tested in Oregon covering the years 1910 through 1946. Only first-calf, 2-year-old records were used. The official records of butterfat production were tabulated separately for cows milked twice a day during 305-day and 365-day lactations. Production of cows milked three times daily, part or all of the milking period, was reduced to a 2-times a day milking basis by using the factor 0.0655 of 1 per cent for each day the cow was milked 3 times. The distribution of the 2690 records between breeds was 1881 Jerseys, 358 Guernseys, 301 Holstein-Friesians and 150 Ayrshires. The number of records available from the College herd was 359, while 2331 were from private herds.

An analysis of variance (6) was applied to the data to find out the significance of the difference in butterfat production of cows calving each month of the year.

RESULTS AND DISCUSSION

Information on the butterfat records of first calf heifers used in the study is given in table 1.

Table 2 gives a summary of the results of the statistical analysis of the data. The variations in butterfat production among cows freshening within each month

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TABLE 1
Average yearly butterfat production of five groups of first calf heifers

Month of fresh-ening	Group 1 ^a		Group 2		Group 3		Group 4		Group 5	
	No. of heifers	Av. prod.	No. of heifers	Av. prod.	No. of heifers	Av. prod.	No. of heifers	Av. prod.	No. of heifers	Av. prod.
		(lb.)		(lb.)		(lb.)		(lb.)		(lb.)
January	80	455	34	372	66	488	19	483	42	320
February	78	464	38	390	68	489	23	479	28	295
March	91	469	32	375	64	474	20	477	24	330
April	102	421	26	379	76	473	11	467	25	304
May	72	425	27	380	81	464	13	479	27	372
June	53	438	18	359	62	465	10	458	19	326
July	48	434	26	338	47	458	18	471	22	326
August	69	459	19	384	78	482	23	467	33	322
September	104	446	19	397	105	466	35	472	49	312
October	70	444	25	398	84	450	24	470	29	341
November	64	461	25	389	69	484	9	459	31	329
December	78	445	29	387	72	498	27	474	30	305
Total & mean	909	447	318	379	872	474	232	473	359	323

^a Group 1. Jersey, 305 day, Register of Merit

Group 2. Guernsey, Holstein, Ayrshire, 305 day, Advanced Registry

Group 3. Jersey, 365 day, Register of Merit

Group 4. Guernsey and Holstein, 365 day, Advanced Registry

Group 5. Ayrshire, Guernsey, Holstein and Jersey herd test (college)

were large, and a definite trend was not followed when the monthly averages were studied, but rather an up-and-down line. Jersey cows milked for 305 days were

TABLE 2
Analysis of variance of the butterfat records used in the study

Group	Source of variation	Degrees of freedom	Sum of squares	Variance	Variance ratio	Significance level	
						5%	1%
1 Jersey R. of M. 305 days	Month	11	209,932.14	19,084.74	2.25	1.80	2.26
	Error	897	7,594,314.99	8,466.35			
	Total	908	7,804,247.13				
2 Guernsey Holstein Ayrshire A.R. 305 days	Month	11	77,933.46	7,084.86	1.45	1.82	2.31
	Error	306	1,496,925.13	4,891.91			
	Total	317	1,574,858.59				
3 Jersey R. of M. 365 days	Month	11	160,275.05	14,570.46	1.02	1.80	2.26
	Error	860	12,275,470.21	14,273.80			
	Total	871	12,435,745.26				
4 Guernsey Holstein A.R. 365 days	Month	11	8,700.71	790.97	0.09	1.83	2.34
	Error	220	2,009,556.38	9,134.35			
	Total	231	2,018,257.09				
5 All breeds College Herd Test	Month	11	124,238.84	11,294.44	1.71	1.81	2.29
	Error	347	2,297,328.15	6,620.54			
	Total	358	2,421,566.99				

the only group that showed significance at the 5 per cent level, although not significant at the 1 per cent level. Since the other four groups showed insignificant differences between the production of cows freshening in different months of the year, the significance of the first group is of doubtful value.

SUMMARY

The butterfat records of 2690 first-calf heifers in herds located in western Oregon, a region with rather uniformly mild temperature, were studied to determine the effect of the month of calving on yearly butterfat production.

It seems that under western Oregon conditions the season of the year in which a cow freshens has no appreciable effect on her yearly butterfat production.

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SOME FACTORS INFLUENCING THE MALE HORMONE CONTENT OF COW MANURE¹

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Riley and Hammond (8) discovered that the feeding of dried cow manure to day-old chicks caused marked stimulation of the comb growth. Evidence was presented indicating that the factor present was an androgenic rather than a gonadotrophic substance. They reported that "feces from bulls were entirely without effect, whereas feces from pregnant cows, as well as from unbred heifers, had marked androgenic effects."

Turner (10, 11, 12) confirmed the report of the presence of orally-active androgens in the feces of lactating cows when dried at 45° C. The androgen content of the manure of other ruminants, including goats and sheep of both sexes and conditions, was either low or absent. The feces of dairy bulls showed indications of only small androgen excretion by that route.

Gassner and Longwell (1, 4) reported that the concentration of androgens in feces reached a peak during the last week of pregnancy and then dropped sharply to zero at calving. Steer and bull manures were relatively inactive biologically.

The present studies were initiated to throw further light upon the functional relationship between the male hormone eliminated in the feces of dairy cows of the several breeds and reproduction and lactation. Further, in connection with studies concerned with the characterization of the androgens excreted and with methods for their extraction, it was considered helpful to know when the greatest concentration of hormone might be expected.

EXPERIMENTAL PROCEDURE

The fresh manure was collected from individual cows of the Guernsey, Holstein and Jersey breeds in the University of Missouri dairy herd. Complete samples were not collected, rather the feces dropped during the milking period in the morning or afternoon were combined until a sufficient quantity was collected for an assay. This usually required 2 to 3 days. Cows in various stages of lactation and pregnancy were included. When a series of samples from the same cow was collected, at least a month intervened between samples. Each collection of fresh manure was placed quickly in a Freas electric drying oven maintained at a temperature of 45° C. Samples were stirred daily. At least 48 hours were required to dry the collection. The dried manure was placed in a large lard can and, when collection was complete, the entire sample was ground in a small hammer mill and thoroughly mixed before assay.

The androgen content of each sample was assayed biologically by the method

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previously described (12). Groups of about 20 sexed White Plymouth Rock chicks were used throughout and the dried cow manure was fed uniformly at the 10 per cent level by substituting the dried manure for an equal weight of alfalfa meal in the basal chick starter ration. These assays were conducted monthly throughout the year.

Each month a group of control chicks and a group fed methyl testosterone at the rate of 20 mg./kg. of feed were included to determine the possible seasonal variation in the responsiveness of the chick comb to androgen. Since no seasonal trend was observed in the average comb weight of either sex in the control group or those fed methyl testosterone, it was decided that no correction factor for season was required (13).

As a measure of the presence of biologically active androgens in the samples, the average comb weight per 100 g. body weight of each sex was determined. The average comb weights per 100 g. body weight for the two sexes were added together and divided by two. This value should represent the average comb weight per 100 g. body weight of a population of chicks containing equal numbers of the two sexes.

As a measure of the biological activity of the male hormone present in the various samples of dried cow manure, comparison may be made with the average comb weight of groups of control chicks of the same age and with groups of chicks fed 10 and 20 mg. of methyl testosterone per kilogram of starter feed. Since the biologically active androgenic hormones present in cow manure are not known, it seems preferable to indicate differences in the various samples in terms of average comb weight rather than in terms of any single androgen. For a comparison of the oral effectiveness of several androgens in fowls the reader is referred to a paper by the writer (14). The average comb weight of all the chicks fed samples of dried cow manure assayed in this study is presented for comparison (table 1).

RESULTS

Effect of pregnancy upon androgen excretion. In the dairy cows available for our study, no clear-cut separation of pregnancy and lactation could be made, since the heifers pregnant for the first time were not stabled. The group of cows included in table 2 were all lactating cows, but they were classified on the basis of the month of pregnancy. Since lactating cows are not bred until 90 days or more after parturition, the group of cows whose manure was assayed during the first month of pregnancy included cows lactating an average of 124 days. By the eighth month of pregnancy, most of the cows had been dried up; only two of eight cows still were lactating slightly. The cows in the ninth month of pregnancy all were dry.

It will be seen that, aside from the second month, the assay results did not vary greatly from month to month until the eighth and ninth months of pregnancy. Apparently there is a tendency for the male hormone excretion rate to increase at the approach of parturition. This rise occurred at a time when the cows were either dry or almost dry.

TABLE 1
Comparison of comb weight of chicks fed control feed, methyl testosterone and cow manure

Treatment	Male chicks				Female chicks				Male + female 2	Ratio of control group
	No. of chicks	Av. body weight	Av. testes weight	Av. comb weight	Comb weight 100 g. body wt.	No. of chicks	Av. body weight	Av. ovary weight	Av. comb weight	Comb weight 100 g. body wt.
Control	185	(g.) 242.4	(mg.) 48.88	(mg.) 102.69	(mg.) 42.37	199	(g.) 224.8	(mg.) 48.61	(mg.) 51.17	(mg.) 22.76
10 mg. MT ^a /kg.	52	248.4	42.86	240.80	96.94	46	236.3	45.45	246.20	104.18
20 mg. MT ^a /kg.	157	239.2	43.61	360.50	150.74	158	226.2	49.78	232.67	111.70
10% cow manure	872	251.8	31.47	202.25	80.31	855	236.1	38.10	137.66	58.31

^a MT = Methyl Testosterone

TABLE 2
Effect of the stage of pregnancy upon the androgen excretion of dairy cattle (assay with white rock chicks—23 days of age)

Month of pregnancy	Lactation days, av.	No. of cows (breed) ^a	Male Chicks				Female Chicks				Male + female $\frac{\quad}{2}$		
			No. of chicks	Av. body weight (g.)	Av. testes weight (mg.)	Av. comb weight (mg.)	Comb weight $\frac{\quad}{100 \text{ g. body wt.}}$ (mg.)	No. of chicks	Av. body weight (g.)	Av. ovary weight (mg.)		Av. comb weight (mg.)	Comb weight $\frac{\quad}{100 \text{ g. body weight}}$ (mg.)
1	124	3(2H, 1J)	32	235.1	28.85	147.24	62.63	43	232.6	33.74	106.19	45.65	54.14
2	169	7(5H, 1G, 1J)	64	248.9	29.93	233.48	93.80	67	236.3	38.87	195.93	82.92	83.36
3	180	6(5H, 1J)	69	243.4	32.95	175.53	72.12	48	224.2	51.14	137.18	61.19	66.66
4	216	4(4H)	34	257.4	37.72	153.73	59.72	46	243.9	45.25	111.33	45.65	52.69
5	284	5(2H, 3G)	56	266.1	40.10	161.61	60.73	47	242.2	46.67	102.10	42.16	51.44
6	286	5(2H, 3J)	49	260.5	33.30	210.05	80.63	51	236.6	66.09	124.19	52.49	66.56
7	294	3(2H, 1J)	29	248.0	28.47	148.70	59.96	29	243.6	39.90	142.10	58.33	59.15
8	Almost dry	8(5H, 1G, 2J)	73	234.1	28.45	281.66	120.32	83	224.2	40.41	190.05	84.78	102.55
9	Dry	7(4H, 3J)	73	272.4	32.12	256.81	94.27	69	252.2	37.88	169.31	67.13	80.70
Non-pregnant, dry cows			33	252.6	28.15	205.81	81.48	43	253.3	38.93	168.53	66.53	74.01
Breed													
Holstein	22		226	249.0	32.04	164.63	66.12	219	235.4	43.52	126.40	53.70	59.91
Guernsey	4		49	267.6	37.68	202.67	75.74	38	250.5	45.61	123.66	49.36	62.55
Jersey	7		58	249.4	27.03	233.96	93.81	74	233.6	54.36	165.85	70.99	82.40

^a H = Holstein, G = Guernsey and J = Jersey.

Whether the apparent high level of androgen excretion during the second month is significant is not clear. One of the Holstein cows appeared to excrete an unduly large amount of androgens at this time in comparison with her other assays. Furthermore, since the tabulation according to the stage of lactation shows no similar increase, the writer prefers to believe that this does not represent a general increased level of androgen excretion.

In order to interpret the fluctuation in the androgen excretion rate from month to month during pregnancy due to possible breed variation, the data were classified on the basis of the breed for the first 7 months of pregnancy. The eighth and ninth months were excluded due to possible effect of the preparturient rise in the androgens. This tabulation indicates little difference in the excretion of androgens by Holstein and Guernsey cows, but the Jersey cows appear to excrete greater quantities of androgens under similar conditions.

A small group of non-pregnant dry cows also is included. The relatively higher androgen excretion rate by this group, as compared to the pregnant group, is believed to be due to the presence of a predominant number of Jersey heifers. It would appear that neither pregnancy nor lactation is necessary for the excretion of relatively large amounts of androgens. This confirms the report of Riley and Hammond (8).

Effect of lactation upon androgen excretion. The data on the individual cows were tabulated according to the stage of lactation (table 3). It will be seen that no trend in the average comb weight with the advance of lactation is present during the first 8 months. Comb weight values above normal in the ninth and eleventh months of lactation are interpreted as indications of the prepartum rise in androgen excretion rather than relationship to the advance in the period of lactation.

The tabulation of the data by breeds, up to the time of the preparturient rise, again indicated little difference in the androgen excretion by Holstein and Guernsey cows. The Jersey cows, however, again were higher but not quite as high as in the tabulation of pregnant animals.

DISCUSSION

While the data are limited, they indicate that a relatively high average level of androgen excretion occurs in unbred heifers and non-pregnant, non-lactating cows. Since this observation is in agreement with that of Riley and Hammond (8), it would appear that this hormone is excreted at relatively high levels in sexually mature heifers without reference to pregnancy. Whether there is a cyclic variation in the androgens in relation to the period of heat in heifers has not been investigated. Since there is much experimental work indicating that androgens can stimulate the growth of the mammary duct system, the cyclic growth of the pubertal duct system of heifers may be due, in part, to the presence of androgens as well as estrogens in the blood at this time.

Following conception, the rate of androgen excretion is not believed to increase markedly. It is true that these data show a high level during the second month of pregnancy with a reduction until the seventh month. Further data

TABLE 3
Effect of the stage of lactation upon the androgen excretion of dairy cattle (assay with white rock chicks—28 days of age)

Month of lactation	Stage of pregnancy av.	No. of cows (breeds ^a)	Female Chicks				Male Chicks				Male + female <div>2</div>	
			No. of chicks	Av. body weight (g.)	Av. testes weight (mg.)	Av. comb weight (mg.)	Comb weight <div>100 g.</div> body wt. (mg.)	No. of chicks	Av. body weight (g.)	Av. ovary weight (mg.)		Av. comb weight (mg.)
1	open	8 (6H, 1G, 1J)	86	263.2	33.48	205.85	78.21	72	234.6	44.21	127.46	54.33
2	open	8 (3H, 1G, 4J)	73	238.2	30.36	151.05	63.41	70	230.8	46.40	93.05	40.32
3	<div>3 open</div> <div>2 bred</div>	5 (4H, 1J)	52	261.7	32.56	206.27	78.82	51	251.1	47.25	128.58	51.21
4	<div>3 open</div> <div>2-29 days</div>	6 (3H, 1G, 2J)	54	262.5	30.55	205.68	78.35	62	234.2	37.05	149.69	63.92
5	<div>4 open</div> <div>2-31 days</div>	6 (5H, 1G)	74	256.1	36.87	204.55	79.87	50	238.8	47.73	133.99	56.11
6	<div>1 open</div> <div>5-67 days</div>	6 (4H, 1G, 1J)	61	249.6	31.86	206.65	82.79	58	220.5	49.40	134.94	61.20
7	<div>2 open</div> <div>2-76 days</div>	4 (4H)	33	269.3	40.75	183.79	68.25	45	239.3	41.58	117.17	48.96
8	116	6 (4H, 1G, 1J)	59	257.1	35.76	179.51	69.82	58	234.8	50.93	93.19	39.69
9	137	7 (4H, 1G, 2J)	70	233.3	28.92	215.99	92.58	67	235.1	39.98	173.83	73.94
10	164	3 (1H, 1G, 1J)	31	267.3	36.03	178.73	66.86	30	244.4	43.26	127.50	52.17
11	217	4 (3H, 1G)	44	256.3	28.10	324.40	126.41	30	232.0	34.68	286.09	123.30
Breed												
Jersey	all	33	374	251.6	34.62	184.49	73.33	364	236.1	50.68	126.34	53.51
Holstein	all	6	93	269.3	34.67	200.47	74.44	76	247.7	44.17	110.01	44.41
Guernsey	all	10	167	255.3	28.93	212.65	83.29	166	234.9	41.44	139.72	59.46

^a H = Holstein, G = Guernsey and J = Jersey

will be required to indicate whether the rise during the second month is significant. Until that time, it seems preferable to believe that early pregnancy is not a period of increased androgen excretion.

It is well known that the first half to two-thirds of pregnancy is a period of rapid duct and lobule-alveolar growth of the udder. This growth is stimulated by the hormone of the corpus luteum, called progesterone, acting upon the anterior pituitary thus stimulating the secretion of the mammogenic hormone. It has been proved that the estrogenic hormones augment the action of progesterone and mammogen. It also has been shown that certain androgenic hormone derivatives can stimulate slight lobule-alveolar mammary growth (7). The fact that the androgenic hormones are not excreted in increased amounts during the first two-thirds of pregnancy suggests that they do not play a predominant role in the great growth of the udder at this time. They may supplement the progesterone and balance physiologically the increasing secretion of estrogen.

The most striking change in the androgen excretion rate occurs during the period preceding parturition. It is well known that the excretion of estrogen both in the urine (15) and feces (2) increases rapidly at the approach of calving in dairy cattle. It is possible that the rise in androgen excretion at this time indicates a mechanism designed to counter-balance or offset, in part, the physiological effect of the rapidly rising prepartum estrogen secretion. It is believed that the secretion of progesterone may decline at this time, thus permitting estrogen to become predominant and to initiate parturition and, by stimulation of the pituitary, to increase the secretion of the lactogenic hormone (5, 6).

Since estrogen has been shown to stimulate the secretion of adrenocorticotrophic hormone by the pituitary, there would be expected increased gluconeogenesis of protein and resultant loss of nitrogen in the urine due to the hormones of the adrenals (3, 9). The androgens are known to have the opposite effect, increasing the retention of nitrogen and body growth by reduction of the secretion of the adrenal cortical hormones (16).

The concurrent rise in both estrogen and androgen during late pregnancy may indicate the presence of an adaptive mechanism of the body by which certain effects of one hormone can be balanced by the opposite effects of the other yet permitting necessary stimulation to prevail, *i.e.*, the estrogen stimulation of the lactogenic hormone.

The rise in androgen secretion at the approach of parturition suggests the need of further study of the relation of estrogen to androgen in the stimulation of the lactogenic hormone. It has been shown that androgenic hormones are capable of stimulating an increase in the lactogenic hormone of the pituitary (5). Does the androgen secreted prepartum supplement estrogen in the stimulation of the lactogenic hormone?

Since the level of excretion of androgenic hormones during most of lactation is rather uniform, there is no reason to believe that the androgens play a dynamic role in the maintenance of milk secretion.

SUMMARY AND CONCLUSIONS

1. Manure from cows of the Guernsey, Holstein and Jersey breeds during various physiological states has been dried at 45° C. and assayed biologically for its content of male (androgenic) hormone.

2. It was observed that sexually mature non-pregnant heifers excrete male hormone at a level comparable to those of mature cows.

3. During the first two-thirds of pregnancy, no tendency for a rise in androgen excretion was observed. There was evidence of a preparturient rise in androgens.

4. With the advance of lactation, no change in androgen excretion was noted except when associated with the approach of the subsequent parturition.

5. Dried cow manure from the Guernsey and Holstein breeds appeared comparable in biological activity; the Jersey cows appeared to excrete slightly more male hormone.

6. It is suggested that the preparturient rise in androgen may be related to the marked rise in estrogen at the same time.

7. It is possible that androgens as well as estrogens play roles in the stimulation of the secretion of the lactogenic hormone by the pituitary at the time of parturition.

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THE INFLUENCE OF THE RATION AND RUMEN INOCULATION ON THE ESTABLISHMENT OF CERTAIN MICROORGANISMS IN THE RUMENS OF YOUNG CALVES¹

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INTRODUCTION

Previous investigations concerning the etiology of digestive disturbances in young calves included a study of the microorganisms which appeared in their rumino-reticular cavities (7). It was observed at that time that rumen fauna and certain characteristic flora similar to those seen in samples from mature animals were not established in the majority of the calves examined until they were several weeks old. Upon direct inoculation of organisms from cows into the rumens of a few calves, the organisms became established in some of them. These calves, which were on various rations, were among those in the herd at that time which progressed most satisfactorily, but proper controls were not included. The investigations were continued for the purpose of attempting to determine if there was any material advantage in stimulating the development in calves of early rumen activity comparable to mature animals.

Limited studies with a few young calves indicated that certain microorganisms characteristic of the rumen flora and fauna failed to become established regardless of how often inoculations were made when most of the dry feed ingested was grain. It generally was possible, on the other hand, to establish these particular rumen microorganisms in calves even before they were a week old, provided they were ingesting good quality hay and no grain. Variations in rumen flora which were related to the feed ingested have been reported for sheep by Elsdon (4), who also cited van der Wath's findings on the same subject. Phillipson (6) also makes reference to this variability of the flora associated with ration differences. It would be expected that a similar situation would exist as regards young calves.

As a result of the preliminary investigations, it was decided to place 4-day-old calves on various systems of feeding, both with and without rumen inoculations, in order to study further the significance of the previous observations. Clinical studies and repeated examinations of the rumen flora and fauna were carried out on these calves during their first 6 weeks of age. Blood plasma vitamin A, carotene and ascorbic acid determinations were made at frequent intervals on many of the calves used in this experiment. The results are reported elsewhere (5).

METHODS

Young calves of both the Jersey and Holstein breeds which had received colostrum usually for 3 days were placed on twice-a-day pail feeding of pasteur-

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ized milk between their second and fourth days of age. They were fed at the daily rate of approximately 0.9 lb. for each 10 lb. of body weight at birth, but within the limits of a minimum of 5 lb. and a maximum of 10 lb. per day. It was hoped to encourage the consumption of dry feeds at an early age by limiting milk consumption to this comparatively low amount. They were treated variously as regards the dry feeds given them. Calves which received hay had access to it while with their dams or were provided on their first day of age with 1 lb. of green alfalfa hay which occasionally had some grasses mixed in it. This was replenished or replaced frequently so that the calves had fresh hay before them at all times. Grain, when included in the ration, was fed once per day as long as the quantities did not exceed 0.5 lb. per day. It consisted of a 14 per cent protein herd mixture of corn, oats, wheat bran and soybean oil meal.

Rumen inoculations were given to some of the calves by passing pieces of freshly obtained cuds from cows into the posterior of the calves' mouths. The cows were being fed alfalfa hay, grain and silage. The inoculations generally were carried out on the fifth, tenth, fifteenth and twenty-first days of age. Samples were obtained from the fore-stomachs of the calves with stomach tubes, using the syphoning method developed for use with the Colorado rumen lavage tube, and, in some instances, samples were taken at the time of slaughter. No difference as regards flora and fauna was observed in samples obtained from the same calves by the two methods.

Calves which did not receive rumen inoculations were cared for by the same personnel as the others and were housed in the same buildings. However, they were separated from direct contact with the other calves and mature animals by partitions and passageways. A limited effort was made to avoid transferring organisms from one calf to another on milk buckets and other equipment.

The rumen samples were examined under the microscope in the fresh condition, using slides and cover glasses, for the purpose of observing the protozoa. For the most part, Gram stained smears were relied upon for bacteriological purposes. In preparing the slides, some of the thick soupy materials containing particles of feed were included, since Baker (2) has shown that some varieties of bacteria tend to remain rather closely attached to feed particles and may not always be readily visible in the free liquid. Descriptions of microorganisms based on morphological and staining characteristics leave much to be desired. However, this method was considered the most advantageous to use for evaluating rumen bacteriological activity under the conditions of this investigation.

RESULTS

The Microorganisms

The protozoa encountered in the rumen samples from the calves appeared to be similar to those mentioned by others, including several investigators whose observations were cited by Baker and Harriss (3) in their recent review article. All those commonly seen in samples from cows were established readily upon

inoculation into calves which were ingesting suitable feeds including hay and hay-plus-grain rations.

Many bacteria differing in morphology and staining characteristics were visible in the rumen samples. Many of them have been described previously by others including Baker (1, 2) and Baker and Harriss (3). Some varieties of organisms were observed to be noticeably present only when appreciable quantities of hay were being consumed and certain other varieties when grain was the principal dry feed ingested. This does not mean that these particular organisms alone were present under such conditions, but merely that they at least were readily visible in the smears. However, when the proportion of grain consumed was high, a few varieties of organisms including those described herein sometimes would appear to make up the majority of the bacterial population. Proof neither was sought nor obtained that the organisms mentioned were the most important ones in the digestion of the feeds present. They were used in this study

TABLE 1

Classification and description of some calf rumen flora observed to vary with the type of feed eaten

Hay Flora ^a	
Group I	Quite large G+ coccoids in closely knit pairs
Group II	Large G+, thick, fairly square-ended rods
	Very large G- cigar-shaped rods
	Smaller G- short rods in fours and multiples of 4
Grain Flora ^a	
Group I	Medium-sized, comparatively thin, G+ rods (sometimes granular stain and variable length)
Group II	G- rods resembling coliform

^a Flora which appeared to be characteristically associated with the ingestion of these feeds.

as indicators of the presence or absence of characteristic bacterial populations. As reported elsewhere, further studies showed that the same organisms possibly might be used as indicators, within certain limits, of the relative ratios of grain and hay being ingested by young calves (8).

It was possible to subdivide the varieties of the organisms which were noticed to be associated with hay consumption into two sub-groups (table 1). The first consisted of large Gram-positive coccus organisms in closely knit pairs and sometimes in groups of four or more, but not in chain formations. The approximate size of the pairs was 2.8×2.3 microns, and some groups composed of more than a single pair were as much as 4.4×4.0 microns. They possibly were similar to those called large sarcina packets by Baker (1). Among the organisms included in the second hay sub-group were large Gram-positive, thick, rather square-ended rods whose length varied between 3.2 and 5.5 microns and which were approximately 2.5 microns wide. They were observed quite frequently in pairs. Gram-negative, extremely large, cigar-shaped organisms, which often were as much as 21.5 microns long and approximately 4.0 microns wide, also were included in this sub-group. It is probable that these latter organisms are similar to those referred to by others as giant ellipsoidal forms (3) or *Ocillospira* (1, 2). Besides

these, in this group were Gram-negative rods of approximately 1.0×0.8 microns in size that tended to group in fours and multiples of four in shapes suggestive of window panes.

Organisms associated with grain consumption also could be sub-divided into two groups (table 1). The first consisted of Gram-positive rods which ranged between 1.7 and 3.4 microns in length and were approximately 0.8 microns in width. They appeared to resemble lactobacilli (10). Present in some smears were masses of either short varieties of this organism or a different organism of similar Gram staining property. Sometimes, especially when considerable numbers were present, a tendency existed for these organisms to stain in a granular manner. The second sub-group were Gram-negative and morphologically resembled coliform organisms or did not differ much from them.

Photomicrographs of the various organism types are reproduced in figure 1.

Variations in the establishment of microorganisms

Calf group 1 (hay plus rumen inoculation). Protozoa and bacteria of the two groups noted to be associated with hay ingestion were established in the ru-

TABLE 2

The influence of the ration and rumen inoculation on the establishment of certain microorganisms in calf rumens

Calf group	Ration	Age	No. calves examined	No. calves protozoa present	No. calves hay flora present		No. calves grain flora present	
					Group I	Group II	Group I	Group II
(wks.)								
I	Hay plus inoculation	3	8	8	8	8	0	0
		6	7	7	7	7	0	0
II	Hay, uninoculated	3	8	0	8	3	0	0
		6	6	0	6	5	0	0
III	Hay and grain plus inoculation	3	7	6	2	1	2	0
		6	7	7	6	2	4	0
IV	Hay and grain, uninoculated	3	5	1	2	2	4	1
		6	5	1	2	2	5	3
V	Calf starter, uninoculated	3	4 ^a	0	0	0	4	4
		6	2 ^a	0	0	0	2	1

^a 2 Calves received hay.

mens of eight calves, which had good quality alfalfa hay available to them from birth, before they reached the age of 3 weeks. The organisms still were present several weeks later in the seven calves which were continued on the experiment. "Grain-type" organisms were extremely scarce in samples from these calves (table 2).

Calf group 2 (hay without rumen inoculation). Protozoa failed to make their appearance in samples from eight similarly-fed but uninoculated calves up to the time they reached the age of 3 weeks and up to 6 weeks in the case of the six of them that were continued on experiment. "Hay flora" of the paired coc-

cus type developed in all eight calves by the time they were 3 weeks old and continued to be present in samples from these calves throughout the experimental period. Samples from all but three of the eight calves contained organisms of the second hay sub-group when the calves were 3 weeks old. By 6 weeks of age, samples from five of the six calves contained some of at least one of the organisms of this sub-group. Organisms of the two grain types also were extremely scarce in samples from these calves (table 2). A fairly large Gram-positive rod, thicker and more tapered at the ends than the one seen in association with grain feeding, was observed in great numbers in samples from two of these calves by the time they were 3 weeks old and in five of the six at 6 weeks of age. These organisms never were observed in appreciable numbers in samples from any of the other calves.

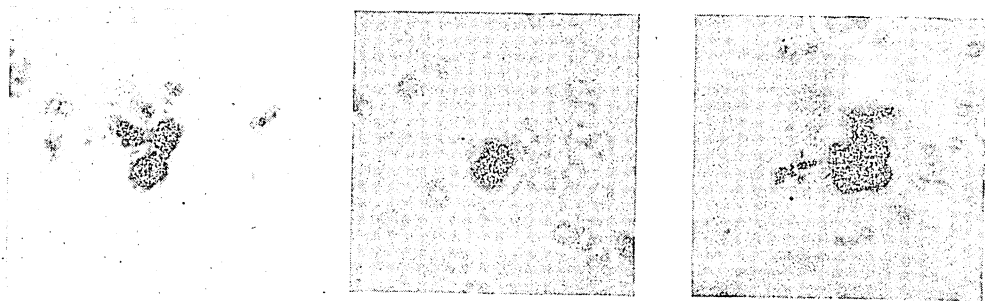
Calf group 3 (hay, grain, plus rumen inoculation). Seven inoculated calves were fed good quality alfalfa hay from birth and a simple 14 per cent protein grain mixture starting on the 14th day of age, both free choice. Protozoa became established in six, hay flora of the paired coccus organism sub-group in two, and hay flora of the second hay sub-group in one, by the time the calves were 3 weeks of age. By 6 weeks of age, samples from all seven had protozoa present, six contained hay flora of the first sub-group and two of the second. Gram-positive grain-type flora developed in two calves by 3 weeks and in four calves by 6 weeks of age (table 2).

Calf group 4 (hay plus grain without rumen inoculation). Only one of five uninoculated calves on a schedule of hay and grain similar to group III developed rumen protozoa by 4 weeks of age or even by 6 weeks of age. Flora of both hay groups were present in samples from two of the five calves by the time they were 3 weeks old but had not yet appeared in the other calves at 6 weeks of age. Bacteria of the first grain sub-group were visible in samples from four calves at 3 weeks of age and in all five calves at 6 weeks of age. Gram-negative bacteria of the second grain sub-group were observed in samples from one calf at 3 weeks and from three calves at 6 weeks of age (table 2).

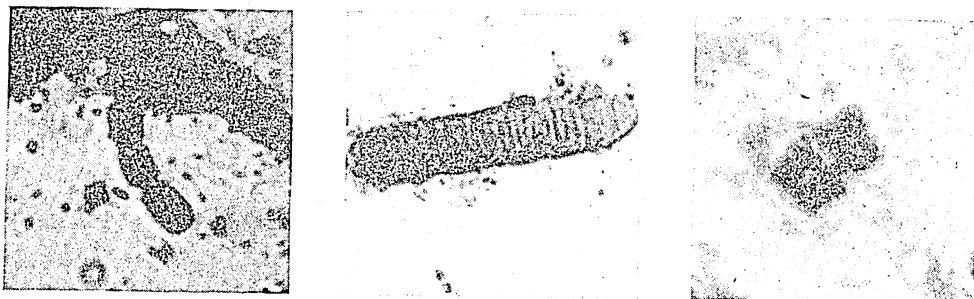
Calf group 5 (calf starter grain ration without inoculation). Four uninoculated calves were fed a commercial calf starter grain ration in pellet form. Two of them received good quality alfalfa hay in addition. Both feeds were given free choice starting on the fourth day of age. The amount of milk fed one of the two calves receiving hay gradually was reduced as the calf increased its consumption of the grain. At 3 weeks of age, no protozoa or hay-type bacteria could be seen in samples from any of these four calves. However, great numbers of the grain-type organisms were present almost to the exclusion of all other bacteria. Only the two calves receiving hay were continued on experiment beyond 3 weeks of age. A similar condition was noted in them when they were 6 weeks of age, although the Gram-negative bacteria resembling coliform organisms appeared to be less prevalent (table 2).

Samples from approximately 20 calves of similar age and on rations fairly similar to those used for the last two groups had been examined repeatedly the previous year. The results were very much the same.

Calf group 6 (shavings and straw bedding without inoculation). Neither protozoa nor hay-type flora were established in any one of seven uninoculated calves which were fed milk alone and bedded with shavings or straw up to the time they were 3 weeks of age. It also was questionable whether any of the



Hay Group I Organisms
(pairs and multiples of pairs)



Hay Group II Organisms
(thick rod, cigar-shaped rod, and group of small rods)



Grain Group I Organisms
(medium-sized rods)

FIG. 1. Photomicrographs of calf rumen microorganisms
(approximately $\times 1800$)

grain-type flora were present in the samples. In fact, relatively few organisms of any kind were present even though all calves ate some of the bedding. Medium-sized, spindle-shaped bacteria which often contained spore-like bodies were present in appreciable numbers in samples from five out of six of these calves

which were bedded with shavings. These were the only ones in which this organism was ever observed.

Growth of the calves. An effort was made to have the two breeds about equally represented in each group with the exception of group VI, which was composed entirely of Jerseys. The average gains in weight made by the calves in all six groups were between 7 and 9 lb. per calf for the first 3 weeks. The calves in groups I, II, III and IV made an average gain of 15 to 16 lb. per calf during the second 3 weeks, while those in group V gained an average of 22 lb. Group VI was discontinued at the end of the first 3 weeks. These gains are lower than the standards given for calves of comparable breeds by Ragsdale (9). Although gain in weight was desired, it was not an objective in these experiments.

Health of the calves. The hair coats of calves on hay alone without rumen inoculation (Group II) appeared to be rougher than those on similar feed which received the inoculation (Group I). Any difference resulting from inoculation was less noticeable or non-existent between the groups of calves receiving rations containing grain. As reported elsewhere (5), calves in group I receiving hay and inoculation maintained on the average uniformly higher blood plasma levels of ascorbic acid during their first 6 weeks of age than any other group. The clinical manifestations of sickness were limited to digestive tract upsets. No trouble was experienced in this respect among the calves in groups I and II, although two calves in each group had rather soft feces on a single day each. The incidence of diarrhea among the calves in group III was 57 per cent, group IV 66 per cent, group V 75 per cent and for group VI 70 per cent. The duration of individual attacks ranged between 2 and 8 days.

Sources of the organisms other than from rumen materials. The organisms designated as Hay Group I and those associated with grain established themselves more readily by natural means in the young calves than did the protozoa and the organisms in Hay Group II. This indicates greater availability of sources of the former. Feces would be a logical source of organisms, augmenting the organisms spread through slobbers from cud-chewing mature animals. Baker (2) examined the feces from a bovine fed on hay and various concentrate rations for some of the characteristic organisms. He concluded that the majority of these organisms were destroyed on passage through the intestines. This seemed to be true based on our Gram stain examinations of fecal samples. However, limited numbers of organisms resembling those designated as Hay Group I and also the Gram-positive varieties of those associated with grain ingestion were present in seven fecal samples from rumen inoculated calves on hay and milk alone. They apparently also were present to a lesser extent in two of four samples from similar calves eating both hay and grain, and in six out of seven samples from cows on mixed rations. Although the varieties associated with grain ingestion were visible in three samples from calves on calf starter ration, no hay-type bacteria could be observed. A young calf was given, by stomach tube, repeated rumen inoculations with feces from a 5-month-old inoculated calf on a hay and skim-milk diet. None of the second-hay-group bacteria or rumen protozoa became established in its rumen even though the first-hay-group organisms did so. Thus,

it would appear that feces from older stock may provide a source from which some of the rumen microflora may be obtained by young calves.

DISCUSSION

It would appear from the present observations that rumen protozoa encounter difficulty in being transferred to young calves under conditions which frequently exist on dairy farms. However, their importance as regards the well-being of the host animals has not been fully established. It is not possible from our data to deduct that they were responsible for the higher blood plasma ascorbic acid levels reported elsewhere (5), or the better appearance of the inoculated calves which received hay alone. In fact, there is more indication that flora were involved because the calves in group I maintained higher blood plasma levels of ascorbic acid than group III, yet both had protozoa in their rumens. Furthermore, a much less satisfactory condition as regards hay-type flora existed in group III as compared to group I.

The type of feed ingested appeared to be the principal factor which influenced the establishment of the organisms designated as group I of those noted to associate with hay and both groups of organisms observed in association with grain ingestion. Evidently the same was partly true of organisms designated as group II of those observed to associate with hay, although the figures indicate a less satisfactory source of organisms for inoculation of the calves than existed for the former. The failure of inoculation to establish hay varieties of flora in calves provided with both hay and grain was unexpected and difficult to explain until later experiments were conducted. These showed that once the ratio of grain ingested exceeded the hay, the proportion of hay varieties of flora appeared to markedly decrease in the Gram stain preparations of rumen samples (8). Thus, it appears that the logical explanation is that some of the young calves tended to eat proportionately more grain than hay when both were offered free choice.

The practical observation that the early development of mature rumen function in young calves may be influenced for the better by inoculation, under some conditions, is probably of some significance. Whether the microscopic observations as outlined here are sufficiently sound and adequately described must await further experimental work under varied conditions. The varieties described as being associated with hay ingestion are sufficiently characteristic in morphology that their recognition probably is quite reliable. However, because of the lack of definite morphological individuality, recognition of organisms designated as associated with grain ingestion possibly is less accurate.

Quite probably the rumen flora may vary somewhat between herds. A slight indication of this has been obtained from examinations conducted on calves in a few other herds and from the reports of others. However, fairly similar conditions probably exist in the majority of herds as regards rumen microorganisms, and observations made on calves in various locations may be comparable.

The comparatively low milk consumption of the calves used in these experiments probably was responsible, especially during the first 3 weeks of age, for

the fact that weight gains were lower than accepted standards (9). The total consumption of milk during the 6-week-period was as low as 210 lb. each for most of the Jersey calves and only two Holsteins received more than 336 lb.

Apparently, rumen inoculation did not influence the ability of the calves to withstand the factors which existed in the herd that stimulated attacks of diarrhea. On the other hand, the type of ration fed, especially good alfalfa hay and milk, appeared to be of more value in preventing the occurrence of this malady. This naturally raises the question as to whether or not, under some conditions, the health of the digestive tracts of young calves may be jeopardized as the result of attempts to make rapid gains in weight at very early ages.

SUMMARY

The rumens of young calves being fed milk and various dry feed rations were inoculated with microorganisms from the rumens of mature stock by placing pieces of cuds from the latter in the posterior of the mouths of the calves. The inoculations were omitted from similarly fed calves used as controls.

The inoculations assisted in the establishment of protozoa in the rumens of calves eating either hay alone or both hay and grain. They assisted in the establishment of some, but not all, of the characteristic varieties of rumen microflora which were associated with hay ingestion in calves fed on alfalfa hay alone. The establishment of varieties of organisms which were associated with the ingestion of grain was not assisted by the inoculations. The establishment of the varieties of flora which were associated with hay ingestion was inhibited in some calves when grain was fed.

The inoculated calves on a diet of alfalfa hay and milk alone were considered to have a better appearance than the controls, but this difference was not apparent between the inoculated and uninoculated groups fed on both hay and grain. Data reported elsewhere (5) show that uniformly higher levels of ascorbic acid in the blood plasma were maintained during the first 6 weeks following birth in the inoculated calves fed alfalfa hay and milk alone than in the calves of any other group. Gains in weight by the calves were very similar in all groups during the first 3 weeks of age. During the second 3-week period, all groups made similar gains except group V, which received a commercial calf starter grain ration. The two groups of calves fed on alfalfa hay and milk alone were free of diarrhea, but the incidence in all other groups was in excess of 50 per cent.

Feces were examined in a search for sources of organisms which resembled natural inhabitants of rumens and some appeared to be present.

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THE INFLUENCE OF THE RATIO OF GRAIN TO HAY IN THE RATION OF DAIRY CALVES ON CERTAIN RUMEN MICROORGANISMS¹

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Variations were observed to occur in certain flora and fauna present in rumen samples from young calves which apparently depended upon the ratio of grain to hay that the calves ingested (1). This indication was pursued further to determine if the observed variations were consistent. Should this be the case, it was hoped that examinations of rumen contents and rumen microorganisms might prove to be more valuable in making differential diagnoses of some calf problems. For instance, information of what feeds have been ingested often is essential in order to determine with any degree of accuracy if a relationship exists between the feed and the unhealthy condition of the calves. Yet, it often is difficult to estimate what the calves actually have been eating when more than one feed has been offered free choice, including the bedding. This problem is complicated further when several calves are fed together in groups because of the individual variations that exist between calves in their choice of feeds.

It perhaps is of interest to add here that extreme variations in rumen microfauna and microflora were observed to be more frequent among young calves fed dry feeds free choice during their first few weeks of age than among similarly-fed older calves. This situation probably resulted from the fact that younger calves ate limited quantities of feed and often limited themselves to only one feed at a time. Because of the relatively small capacity of their rumens, the influence of eating a single feed on the microorganisms was much greater than in older calves in which the buffering effect of larger amounts of previously-eaten feeds existed.

METHODS

The 19 calves used were between 1 and 4.5 months of age. Rumen flora and fauna, which had been classified as quite characteristic, had been present in all the calves prior to the time this particular study was undertaken. Most of them had been inoculated by use of cuds from mature animals in the manner outlined previously (1). It was difficult to determine accurately the relative quantities of hay and grain ingested by calves younger than these and, consequently, data from such calves were omitted.

The quantities of hay and grain consumed during the 4 days prior to the examinations were used in arriving at the relative ratios of the two feeds eaten by the calves. However, they actually were consuming approximately the same proportions for some days longer than this. This period was chosen on the basis of experience with the establishment of characteristic flora and fauna in the rumens of young calves.

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Some calves received different ratios of hay to grain at different times either by design or their own choice and, consequently, data from them were included in more than one category. The hay was fairly good quality alfalfa hay and the grain was a simple 14 per cent protein mixture containing 4 parts of corn, 3 of oats, 1 of bran and 1 of soybean oil meal.

Rumen samples were obtained by use of stomach tubes and examined microscopically in the fresh state and by use of Gram stains as outlined previously (1). The preparations were examined for the same organisms as those discussed in the previous paper (1). Two main groups of bacteria were noted, one associated with hay ingestion and the other with grain, and two sub-groups were recognized in each group.

RESULTS

The results of 35 examinations are summarized in table 1. Column 3 in the

TABLE 1
The influence of the ratio of grain to hay on some calf rumen microorganisms

Ratio			No. rumen samples	No. samples protozoa present	No. samples Hay flora present		No. samples Grain flora present	
Grain : Hay					Group I	Group II	Group I	Group II
0	:	1	19	19	19	19		
1	:	3	6	6 ^b	6	6	1	1
							4 ^a	
2	:	3	4	3 ^b	4	2	4	2
				1		2 ^a		
1	:	1	6	6 ^b	6	3	3	5
						1 ^a		
3	:	2	3	2 ^b	3	2	3	2
				1 ^a				
3	:	1	2	2 ^a			1 ^b	1 ^b
							1	1
1	:	0	5	1 ^a			4 ^b	3 ^b
								1

^a = few; ^b = masses; unmarked = moderate or appreciable numbers.

table shows that protozoa were present in all samples except four of the five from animals on grain without hay. Moderate numbers were observed to be present when the dry feed ration consisted of hay alone. With the addition of some grain to the ration, the numbers of protozoa in the samples increased greatly. This is in agreement with the findings of others as summarized by Phillipson (2). A marked reduction in numbers followed, once the ratio exceeded 3 parts of grain to 2 parts of hay. Limited numbers only were present in samples from calves eating three or more times as much grain as hay. The one calf receiving grain without hay, but still having protozoa present in the rumen, was one of the oldest calves used. It had been fed grain with straw for bedding for 10 days at the time of the examination.

Organisms which were classified as belonging to the hay flora groups were visible in Gram stain preparations of all 19 samples from calves on rations of hay alone. The prevalence of these organisms in the smears appeared to in-

crease with the addition of some grain to this ration. However, as the proportion of grain ingested approached quantities equal to the hay, a reduction was rather apparent. Organisms of the second hay group were reduced more noticeably than those of the first hay group in samples from animals eating as much or more grain than hay. As shown in columns 4 and 5, both groups of the organisms associated with hay were missing from the samples once the ratio reached 3 parts of grain to 1 of hay. Thus, the organisms associated with hay consumption disappeared from the rumen samples at lower ratios of grain to hay than did the protozoa. The apparent increases in the flora associated with hay ingestion on the addition of some grain to rations of hay alone may have resulted from the eating of more balanced rations by the calves. Such is suggested by the observations of Van der Wath, as cited by Phillipson (2), that bacterial numbers were influenced by the diet, with balanced rations being the most satisfactory.

Only limited numbers of the bacteria which were observed to associate with grain rations were visible in the samples until a proportion of 3 parts of grain to 2 of hay was being consumed (columns 6 and 7). These organisms increased in relative prevalence in comparison with other flora as the proportion of grain increased. On rations of grain alone, some samples appeared to contain practically no other organisms.

Very small Gram-negative organisms were noticeably prevalent in samples from calves on rations containing hay alone or high proportions of hay. Small Gram-positive short rods or cocci were observed in increasing proportions on the addition of grain to rations of hay.

Although data collected on this group of calves are very limited, they indicate that by observing certain flora and fauna present in rumen samples from calves, it may be possible to estimate the relative ratio of grain to hay that they are ingesting.

SUMMARY

A total of 35 rumen samples from 19 calves between the ages of 1 and 4.5 months were examined microscopically. The calves received rations of alfalfa hay or grain alone, or various proportions of these. Most of them had received rumen inoculations and the remainder had been exposed to usual rumen micro-organisms in a natural manner.

Moderate numbers of protozoa and flora of varieties observed to associate with hay ingestion accompanied the ingestion of hay without grain.

Masses of protozoa along with fairly numerous flora of the 2 hay groups were associated with the consumption of hay and moderate quantities of grain.

Similar concentrations of protozoa, accompanied by rather limited numbers of organisms of the hay groups and fairly numerous bacteria of the varieties observed to associate with grain consumption, accompanied the ingestion of approximately equal quantities of hay and grain.

Limited numbers of protozoa accompanied by great numbers of bacteria of the grain groups, but no organisms of the hay groups, were present when the ration consisted of almost all grain.

Protozoa and organisms of the varieties associated with hay ingestion generally were absent entirely in samples from calves on strictly grain rations.

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THE INFLUENCE OF THE RATION AND EARLY RUMEN DEVELOPMENT ON THE CHANGES IN THE PLASMA CAROTENOIDS, VITAMIN A AND ASCORBIC ACID OF YOUNG DAIRY CALVES¹

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The period between birth and the time a calf has developed into a normally-functioning ruminant is recognized as critical from the standpoint of nutritional well-being and health. As pointed out by Pounden and Hibbs (8), this period may extend for many weeks in some cases, when judged by the presence or absence of characteristic rumen microorganisms.

Several reports have appeared in the literature showing the usual changes in the blood plasma carotenoids and vitamin A of calves from birth to several weeks of age (1, 2, 3, 6, 11, 12, 14, 15). The changes in plasma ascorbic acid have been reported by Phillips *et al.* (7), Hibbs and Krauss (2), Sutton and Kaeser (12), and Teeri *et al.* (14).

Wise *et al.* (15) have reported results showing that after the blood carotenoids reach a peak on about the third day, as the result of colostrum feeding, there is a rapid decline for from 4 to 5 weeks and then a gradual rise to the post-colostrum feeding level or slightly above at 8 to 10 weeks of age. Vitamin A follows a somewhat similar trend. Teeri *et al.* (14) report that, on the average, blood carotenoid values level off between 15 and 23 weeks of age at about 44 γ per 100 ml. in Holstein calves and at about 65 γ per 100 ml. in Jersey and Guernsey calves. Considerable individual variation is indicated by the high and low values obtained in calves apparently fed and managed alike.

It is striking that until most calves are several months old their blood carotenoid levels do not even approach the levels found in mature animals fed on dry feeds. This may be the result of the ability of the mature animal to consume relatively large quantities of roughage. It is not illogical, however, in the light of our previous observations regarding the variations in the rate of establishment of characteristic rumen microorganisms in calves (8), to assume that the differences in the blood carotenoids and vitamin A levels between calves and adult animals might be due, at least in part, to their relative ability to digest roughage in the rumen. Furthermore, many of the individual variations found among calves may be due, in part, to the differences in the age at which normal rumen function begins.

Investigations were undertaken, therefore, to study the influence of the ration and rumen inoculations on the establishment of rumen function in young calves, the results of which are reported elsewhere (9, 10). Concurrently, a study was made on the influence of the ration and early rumen development on the changes

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occurring in the plasma carotenoids, vitamin A and ascorbic acid of dairy calves during their early postnatal development.

EXPERIMENTAL

Plasma vitamin A and carotenoids were determined by the method described by Kimble (4). The method of Mindlin and Butler (5) was used to determine plasma ascorbic acid.

After a colostrum feeding period, usually 3 days, all calves were fed whole milk for the entire experimental period of 6 weeks at the rate of 0.9 lb. per 10 lb. of body weight at birth. This relatively low level of milk feeding was adopted in order to encourage the calves to consume more of the dry feeds offered.

Beginning on the fourth day of age, calves born in the herd from January until April, 1948 were assigned to one of six groups and fed whole milk and various dry feeds with and without rumen inoculations as follows: Group I, alfalfa hay plus rumen inoculations; Group II, alfalfa hay alone; Group III, alfalfa hay plus grain (14 per cent protein herd ration) plus rumen inoculations; Group IV, alfalfa hay plus grain (14 per cent protein herd ration); Group V, alfalfa hay plus standard calf starter pellets; Group VI, whole milk only (this group was continued on experiment for only 3 weeks).

The rumen inoculations in groups I and III were accomplished by direct transfer of small pieces of cud material to the mouths of the calves from cows in the herd. This was done in order to make certain that these calves had access to the microorganisms normally present in the rumens of the adult animals.

All calves were bled as nearly as possible on the fourth and seventh days of age and weekly thereafter until the forty-second day of age and the plasma carotenoids, vitamin A and ascorbic acid were determined.

The results of the blood analyses are shown in table 1. No beneficial effect of rumen inoculations on the blood plasma carotenoids was observed. Average figures for the calves in groups III, IV and V, which were fed grain, show that when grain was included in the ration, plasma carotenoids did not increase during the first 6 weeks to the extent observed in groups I and II, which were fed alfalfa hay as the only dry feed. The calves in groups I and II made an average steady increase in plasma carotenoids from 4 days until 42 days of age, reaching extremely high levels as compared to any of the other groups.

Plasma vitamin A was found to decrease markedly from the fourth until the forty-second day of age in all groups except group V, which received a calf starter containing 5,000 U.S.P. units of supplemental vitamin A per lb. No marked beneficial effect from rumen inoculations was noted on the plasma vitamin A level although the values for vitamin A appear to be somewhat higher in group III as compared to group IV.

The level of plasma ascorbic acid was found to decline between the seventh and fourteenth days of age in all groups except group I, which was fed alfalfa hay plus rumen inoculations. This group maintained the most uniformly high level of plasma ascorbic acid during the first 4 weeks of any of the groups.

TABLE 1
The influence of the ration on the changes in the plasma carotenoids, vitamin A and ascorbic acid of the blood of young calves

Group ^a	No. of calves	Plasma carotenoids Ages of animals						
		4 days	7 days	14 days	21 days	28 days	35 days	42 days
Plasma carotenoids (γ/100 ml.)								
I	6	32.3 ± 6.8 ^b	34.9 ± 5.5	42.5 ± 4.4	58.2 ± 16.3	76.9 ± 14.2	93.2 ± 20.0	99.2 ± 23.3
II	6	35.9 ± 5.7	38.2 ± 5.8	49.9 ± 14.0	53.6 ± 19.4	67.9 ± 23.9	92.6 ± 20.0	96.6 ± 22.0
III	7	20.6 ± 2.9	20.7 ± 3.1	34.0 ± 7.7	37.0 ± 12.7	32.7 ± 9.1	36.2 ± 10.7	49.7 ± 3.3
IV	5	21.5 ± 3.9	32.7 ± 9.8	36.2 ± 7.5	35.4 ± 13.7	35.2 ± 10.3	44.4 ± 12.3	56.8 ± 15.4
V	4	38.4 ± 20.0	32.7 ± 8.4	35.1 ± 8.9	31.4 ± 10.3	33.0 ± 0.0	35.5 ± 10.7	28.3 ± 2.6
VI	6	19.4 ± 3.5	22.4 ± 4.5	27.5 ± 4.6	20.0 ± 2.6			
Plasma Vitamin A (γ/100 ml.)								
I	6	20.3 ± 3.3	16.4 ± 3.1	14.8 ± 1.5	12.1 ± 0.9	10.8 ± 1.2	9.2 ± 1.8	8.1 ± 1.0
II	6	16.1 ± 2.8	13.7 ± 2.3	11.7 ± 1.5	9.6 ± 2.2	6.8 ± 1.6	7.5 ± 1.1	7.7 ± 1.1
III	7	13.1 ± 1.8	12.2 ± 2.5	11.6 ± 1.3	10.5 ± 1.6	7.9 ± 1.1	8.9 ± 1.5	9.0 ± 1.2
IV	5	13.5 ± 1.4	9.9 ± 1.2	8.6 ± 1.4	8.8 ± 1.0	6.5 ± 0.9	7.0 ± 0.7	6.3 ± 0.3
V	4	15.1 ± 5.3	13.4 ± 4.6	12.8 ± 3.1	11.7 ± 1.8	15.0 ± 0.0	16.0 ± 2.5	14.7 ± 1.8
VI	6	9.2 ± 1.7	7.9 ± 1.3	9.4 ± 0.9	6.3 ± 1.4			
Plasma ascorbic acid (mg./100 ml.)								
I	5		0.47 ± 0.06	0.46 ± 0.03	0.42 ± 0.04	0.47 ± 0.03	0.43 ± 0.09	0.36 ± 0.05
II	5		0.47 ± 0.06	0.27 ± 0.05	0.25 ± 0.01	0.29 ± 0.05	0.46 ± 0.04	0.42 ± 0.03
III	7		0.49 ± 0.02	0.26 ± 0.03	0.38 ± 0.07	0.33 ± 0.04	0.38 ± 0.06	0.41 ± 0.05
IV	5	0.44 ± 0.03	0.36 ± 0.04	0.33 ± 0.09	0.32 ± 0.02	0.30 ± 0.05	0.40 ± 0.08	0.50 ± 0.07
V	3		0.41 ± 0.05	0.30 ± 0.04	0.40 ± 0.00	0.39 ± 0.00	0.30 ± 0.00	0.44 ± 0.00
VI	6	0.53 ± 0.01	0.59 ± 0.03	0.33 ± 0.08	0.35 ± 0.04			

^a Group I. Whole milk plus alfalfa hay plus rumen inoculations.

Group II. Whole milk plus alfalfa hay.

Group III. Whole milk plus alfalfa hay plus grain plus rumen inoculations.

Group IV. Whole milk plus alfalfa hay plus grain.

Group V. Whole milk plus commercial calf starter.

Group VI. Whole milk only.

^b Standard error.

By the fifth week there was very little difference among all the groups. Group II, which received alfalfa hay alone without the rumen inoculations, declined in plasma ascorbic acid to the lowest level of any of the groups during the first 4 weeks. Groups III, IV and V, in which grain was included in the ration, declined sharply but recovered to a level intermediate between groups I and II by the twenty-first day of age.

In view of these results, it was decided to investigate the possible effects of the addition of grain to the ration of older calves which had been fed hay as the only dry feed. Four calves from groups I and II were continued on whole milk plus alfalfa hay to an average of 64 days of age.

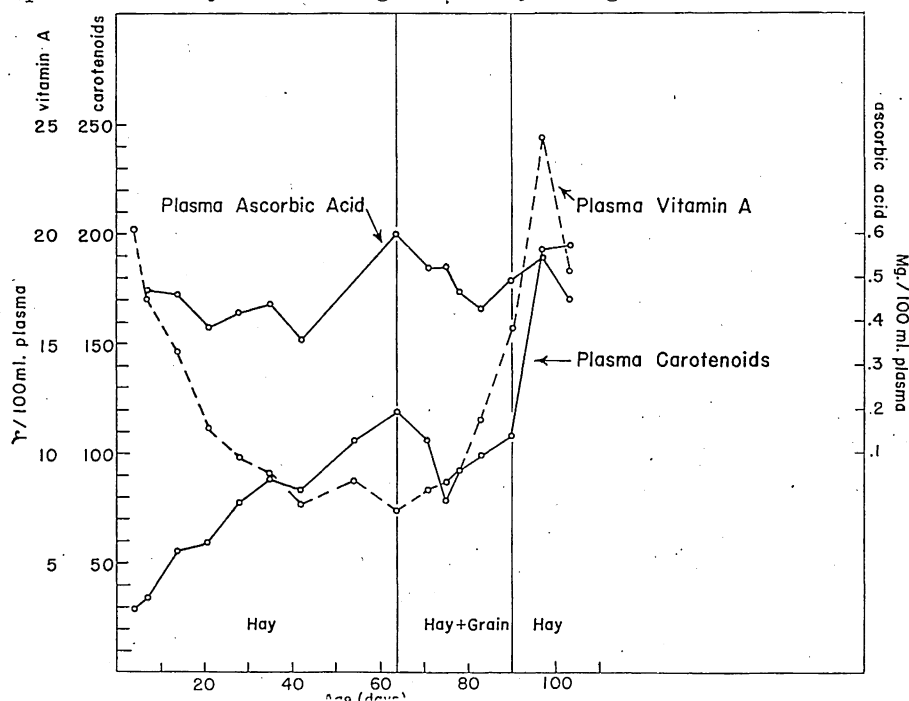


FIG. 1. The influence of adding grain to the ration of 64-day-old calves fed only whole milk and alfalfa hay until that time.

As shown in figure 1, the average plasma carotenoids had increased consistently from 29 γ per 100 ml. at 4 days of age to 118 γ per 100 ml. at 64 days of age. Plasma vitamin A had declined from 20.2 to 7.4 γ per 100 ml. during the same period, and plasma ascorbic acid values had increased from 0.47 to 0.60 mg. per 100 ml.

At this point, a 14 per cent protein grain mixture was included *ad libitum* in the ration. The week prior to the change in ration the calves were eating an average of 2.4 lb. of hay per day. Hay consumption decreased during the grain feeding period. After 4 weeks of grain feeding, the calves were eating 1.7 lb. of hay and 2.6 lb. of grain per day. Grain then was removed from the ration, and during the following week the calves consumed an average of 2.6 lb. of hay per day.

Figure 1 indicates the changes that occurred in the levels of blood plasma carotenoids, vitamin A and ascorbic acid as the result of adding grain to the ration. The plasma carotenoid level was shown to decrease, the plasma vitamin A increased and the plasma ascorbic acid decreased during this period. When grain was removed from the ration, the carotenoids rapidly increased from 108 to 193 γ per 100 ml. within a week, and the plasma vitamin A continued to rise to a peak of 24.3 γ per 100 ml. No marked changes occurred in the plasma ascorbic acid level after the grain was removed from the ration.

DISCUSSION

So far as the blood picture is concerned, the only marked beneficial effect of rumen inoculations appeared to be the higher plasma ascorbic acid level maintained in group I as compared to group II, where alfalfa hay was the only dry feed fed. The mode of action through which this effect was elicited is not readily explainable.

The suppressing action of grain feeding on the blood plasma carotenoids is strikingly demonstrated by the differences in the plasma carotenoid levels between groups I and II, which were fed hay alone, and groups III, IV and V, which received grain in addition to the hay. Accurate records of hay consumption were difficult to obtain during the first few weeks. Therefore, data are not available to demonstrate conclusively whether increased hay consumption or increased digestibility of the hay played the leading role in causing the relatively higher plasma carotenoid level of groups I and II as compared to groups III, IV and V. Indications from the data obtained were that the calves fed grain consumed less hay than those fed hay alone. It would seem that decreased digestibility of the hay possibly was a factor contributing to the low carotenoid levels observed in the grain-fed groups based on the conditions observed with respect to the microorganisms in the rumen when high proportions of grain to hay were fed (10). This would be likely especially when grain consumption reached a level equal to or higher than the hay consumption, as was the case in many instances.

It was noted that the plasma vitamin A level decreased when the plasma carotenoids were increased in groups I and II. The opposite effect on plasma vitamin A was observed when plasma carotenoids declined following the addition of grain to the ration of 64-day-old calves (fig. 1). This suggests that the plasma vitamin A level of the blood is not always a reliable indicator of the state of vitamin A metabolism in the young calf.

Sutton and Soldner (13) have presented data showing that in adult cattle the seasonal changes in blood plasma vitamin A do not closely follow blood plasma carotene changes but tend to lag behind. Plasma vitamin A often was observed to increase when plasma carotene was on the decline.

There are several factors which may be responsible for these apparent discrepancies in the behavior of plasma vitamin A in relation to the plasma carotenoids. Possibly, one of these complicating factors is the liver storage of vitamin A. The degree of saturation of the liver and whether the vitamin A

stores are being increased or depleted may influence the plasma vitamin A level, independent of the effect of the intake of carotene from the roughage. It also is possible that factors affecting the conversion of carotene to vitamin A complicate the blood picture. The answers to these questions must await further work involving the relationship between blood plasma vitamin A and liver storage, the sources of vitamin A and carotene and the physiology of the conversion of carotene to vitamin A in the calf.

SUMMARY AND CONCLUSIONS

Preliminary investigations indicated that the development of the rumen in young calves is influenced by the type of ration fed. Experiments were conducted, therefore, to determine the effect of different rations and early rumen development on the levels of vitamin A, carotenoids and ascorbic acid in the blood of young dairy calves.

Rumen inoculations, accomplished by direct transfer of cud material from cows in the herd to the calves, were supplied to about one-half the calves in order to make certain that they had access to the microorganisms present in the rumens of adult animals.

Rumen inoculations were effective in preventing the usual drop in blood plasma ascorbic acid between the seventh and fourteenth days of age when only alfalfa hay and milk were fed but were ineffective when grain was included in the ration. A ration of whole milk and alfalfa hay alone resulted in carotenoid levels considerably higher after 14 days of age than was observed when grain was included in the ration. Rumen inoculations had no marked effect on the blood carotenoid levels. Neither the inoculations nor the type of ration fed markedly influenced the blood plasma vitamin A.

When grain was introduced into the ration of 64-day-old calves, which had been fed only alfalfa hay and milk until that time, a marked reduction in hay consumption and blood carotenoids resulted. Plasma vitamin A increased and ascorbic acid declined during the same period.

These results, when correlated with the effect of different rations on the development of various rumen microorganisms in these calves, indicate that palatable, high quality hay stimulates the early development of rumen function in the young calf and appears to have a favorable physiological effect in meeting the vitamin needs of these animals.

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THE EFFECT OF SUPPLEMENTAL VITAMIN A UPON GROWTH, BLOOD PLASMA CAROTENE, VITAMIN A, INORGANIC CALCIUM, AND PHOSPHORUS OF HOLSTEIN HEIFERS^{1, 2}

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The nutritional value of vitamin A for dairy cattle has been generally accepted and numerous investigators have studied the minimum vitamin A and carotene requirements of calves and heifers for growth. Boyer *et al.* reported that 75 γ of carotene per kg. body weight was adequate for yearling Holstein heifers. Jones and Haag (5) found that heifers did not require supplemental vitamin A for growth and reproduction if they were pastured during the summer. Keyes *et al.* (6) obtained more gain in body weight by supplementing a standard calf starter with vitamin A. With this in mind, a study was undertaken to determine the value of supplementing one of the commonly used heifer rations with vitamin A. The effect of supplemental vitamin A upon blood plasma carotene, vitamin A, inorganic calcium and phosphorus concentrations and growth was studied.

EXPERIMENTAL PROCEDURE

A preliminary experiment was conducted with 22 Holstein heifers from February 1 to May 24, 1946. These animals were divided into two similar groups based upon age and body weight. Both groups were fed and managed identically except that the vitamin A group received supplemental vitamin A. The vitamin A supplement used in these trials was prepared from a fish liver oil source in linseed oil meal and soybean oil meal in the amount of 250,000 USP units per lb. based on manufacturer's analysis. The basal and experimental rations were prepared with similar composition except for the supplemental vitamin A. The animals were fed mixed hay *ad libitum* and 10 lb. of grass silage and 8 lb. of a grain mixture containing 14 per cent crude protein per day. After April 1 the amount of grain was increased to 10 lb. per day.

Growth was determined by measuring body weight and height at withers of the animals. They were weighed and measured at the beginning and end of the experiment with one intermediate weight taken in April.

Blood plasma carotene and vitamin A were determined at monthly intervals using the methods of Moore (8) and Kimble (7), respectively. Blood plasma inorganic calcium and phosphorus were determined at monthly intervals using

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the Clark-Collip modifications (3) of the Kramer-Tisdall method for calcium and the method of Gomori (4) for phosphorus, respectively.

The main experiment was conducted from January 1 to May 6, 1947, using 28 Holstein heifers. These heifers were divided into two similar groups based upon age, body weight, blood plasma carotene and vitamin A, and blood plasma inorganic calcium and phosphorus concentrations. Both groups were fed and managed identically except that the vitamin A group received supplemental vitamin A in the form used in the preliminary trials. The heifers were fed a weighed amount of mixed hay and grass silage at each feeding. They also received 10 lb. of grain per day.

Growth of the heifers was measured by determining the body weight and height at withers at the beginning and end of the experiment with one intermediate weight in March. Chemical analyses were the same as in the preliminary experiment, except that in the determination of blood plasma carotene and vitamin A the carotene was removed (1) previous to determining vitamin A when over 200 γ of carotene per 100 ml. were present. All data obtained in these trials were treated statistically where applicable (10).

RESULTS

In the preliminary experiment, the 11 heifers in the vitamin A group received an average daily intake of 40,382 USP units of supplemental vitamin A. This resulted in no significant differences in gain of body weight or height at withers between the two groups of 11 heifers each. The control group gained 130 lb. and 4.9 cm. and the vitamin A group gained 135 lb. and 4.9 cm.

In the main experiment the basal ration contained an average of 114,000 USP units of vitamin A per daily allowance per heifer. This was supplemented with 129,400 USP units of vitamin A per day for the 14 heifers in the vitamin A group. An analysis of variance of the gains in weight and height at withers as presented in table 1 showed a significant increase in gain in weight but no significant difference in gain in height at withers resulting from feeding supplemental vitamin A. The vitamin A group of 14 heifers gained an average of 235.9 lb. and 9.4 cm. while the control group gained 187.6 lb. and 8.4 cm., or a difference of 48.3 lb. and 1.0 cm. The heifers in the vitamin A group exhibited better condition, being smoother throughout and showing more flesh. The hair was glossier and smoother, and the hides seemed to be more pliable than those of the control group.

Feeding supplemental vitamin A at either level increased the blood plasma vitamin A concentrations and decreased the blood plasma carotene concentrations of the heifers. The mean blood plasma vitamin A concentrations of the heifers used in the preliminary trial were 18.14 γ per 100 ml. for the control group and 21 γ per 100 ml. for the vitamin A group during this trial. Similarly, during the main trial the mean blood plasma vitamin A concentrations were 15.82 and 21.71 γ per 100 ml. for the control and vitamin A-fed groups, respectively. These differences were highly significant statistically. The data from the main trial are presented in table 2. During the course of this trial, the blood plasma vitamin

A concentrations of the control group increased an average of 1 γ of vitamin A per 100 ml., whereas that of the vitamin A group increased 9 γ per 100 ml. The mean for each group was 14 γ of vitamin A per 100 ml. at the beginning of the trial.

The feeding of supplemental vitamin A resulted in a depression of the blood plasma carotene concentrations. In the preliminary trial, the blood plasma carotene concentration of the vitamin A-fed group decreased from 183

TABLE 1
Growth of heifers during main experiment

Heifer no.	Body weight			Height at withers		
	Initial	Final	Gain	Initial	Final	Gain
	(lb.)	(lb.)	(lb.)	(cm.)	(cm.)	(cm.)
Control group						
729	700	875	175	120	124	4
732	668	800	132	113	120	7
734	749	967	218	120	128	8
737	565	716	151	113	120	7
738	600	800	200	115	121	6
740	513	732	219	112	120	8
742	637	871	234	110	120	10
744	526	675	149	106	117	11
747	513	700	187	106	115	9
749	456	579	123	106	115	9
752	374	622	248	100	110	10
755	404	610	206	101	106	5
756	334	513	179	96	107	11
757	344	550	206	94	107	13
\bar{X}	527	715	188	108	116	8
Group fed vitamin A						
731	766	908	142	121	126	5
733	716	1027	311	117	123	6
735	732	1069	337	116	124	8
736	668	930	262	110	118	8
739	668	970	302	114	120	6
741	555	750	195	108	116	8
743	637	890	253	109	119	10
745	501	700	199	109	121	12
746	489	725	236	105	117	12
748	539	755	216	104	118	14
751	404	615	211	101	111	10
753	384	560	176	99	110	11
754	344	593	249	100	109	9
759	275	489	214	93	106	13
\bar{X}	548	784	236	108	117	9

to 96 γ per 100 ml., whereas the control group decreased from 220 to 153 γ per 100 ml. During the main trial, the blood plasma carotene concentrations decreased from 278 to 156 and from 271 to 85 γ per 100 ml. of blood plasma for the control and vitamin A-fed groups, respectively. Analyses of variance proved this difference to be highly significant statistically.

Blood plasma carotene and vitamin A determinations were continued during June, July and August, while the cattle were on pasture after the preliminary experiment, to determine if there was a carry-over effect from feeding supple-

TABLE 2
The effect of supplemental vitamin A upon blood carotene, vitamin A, calcium and phosphorus

Heifer no.	Carotene			Vitamin A			Calcium			Phosphorus		
	Nov. 26	March 11	May 6	Nov. 26	March 11	May 6	Nov. 26	March 11	May 6	Nov. 26	March 11	May 6
	(γ/100 ml.)			(γ/100 ml.)			(mg./100 ml.)			(mg./100 ml.)		
							Control group					
729	393	175	153	17	19	15	8.80	8.68	8.28	8.10	8.63	7.15
732	650	184	134	13	16	13	9.80	9.18	9.31	9.18	7.94	7.56
734	260	142	140	21	24	21	8.93	8.18	8.68	10.11	8.69	9.18
737	501	232	178	11	20	13	10.17	8.25	8.68	10.39	8.75	8.32
738	375	119	108	18	16	17	9.11	9.05	9.08	9.06	9.12	8.21
740	404	190	170	12	18	27	9.36	9.67	9.03	10.11	9.25	7.35
742	210	246	199	13	18	13	10.11	9.05	9.72	8.81	8.81	6.52
744	175	184	178	12	16	17	9.73	9.36	9.40	9.44	9.30	8.57
747	307	236	167	17	15	18	9.67	8.74	8.91	8.93	10.54	7.93
749	156	187	151	4	13	9	9.67	9.30	9.72	8.57	10.54	7.72
752	94	131	126	16	11	13	9.49	9.55	9.72	8.69	9.63	8.45
755	94	134	151	19	15	14	9.55	9.11	9.66	9.97	10.25	8.93
756	148	151	164	15	14	15	9.24	9.55	8.80	10.04	11.21	9.44
757	123	156	159	14	11	12	9.80	9.67	9.14	9.13	9.57	8.45
\bar{X}	278	176	156	14	16	15	9.53	9.10	9.15	9.32	9.45	8.13
							Group fed vitamin A					
731	634	96	108	17	18	19	8.93	9.05	9.14	9.70	6.76	6.72
733	522	116	112	23	19	18	8.56	8.87	8.51	9.18	7.88	6.81
735	398	100	105	20	22	24	9.73	9.18	9.14	7.46	8.75	6.47
736	466	156	134	17	28	34	9.61	9.61	9.26	7.05	7.88	8.10
739	561	114	85	20	26	40	9.42	9.55	9.08	8.81	7.67	6.95
741	149	112	67	1	15	12	9.61	9.11	9.89	7.25	9.30	7.20
743	183	137	105	13	26	22	9.49	9.30	9.89	8.10	6.86	6.76
745	123	103	76	7	18	19	10.11	9.92	9.77	10.39	9.44	7.72
746	224	98	74	11	18	20	10.11	8.87	8.97	6.81	9.44	6.66
748	123	69	52	7	21	24	9.73	9.49	9.08	9.70	9.57	7.15
751	96	78	63	17	17	23	8.93	8.93	10.23	9.97	9.37	7.56
753	96	33	48	13	13	20	9.42	9.42	9.43	8.57	7.88	7.35
754	105	80	63	16	20	23	9.80	10.04	9.31	10.39	9.18	7.15
759	110	91	94	15	19	30	8.99	8.68	9.49	8.95	9.97	8.93
\bar{X}	271	99	85	14	20	23	9.52	9.29	9.37	8.74	8.57	7.25

mental vitamin A. It was found that the heifers receiving supplemental vitamin A had lower blood plasma carotene and vitamin A concentrations while on pasture than the heifers that did not receive supplemental vitamin A. The mean blood plasma vitamin A concentrations were 22.15 γ per 100 ml. for the control group and 17.45 γ per 100 ml. for the vitamin A group. This difference was significant. The mean blood plasma carotene concentrations were 874 γ per 100 ml. for the control group and 696 γ per 100 ml. for the vitamin A group. This difference was highly significant.

Feeding supplemental vitamin A had no effect upon the blood plasma inorganic calcium and phosphorus concentrations of the heifers. In the preliminary experiment there were no significant differences between the two groups; however, both groups had higher blood plasma inorganic calcium concentrations and lower blood plasma inorganic phosphorus concentrations during the summer pasture period than during the feeding trial. In the main experiment there was no significant difference in the blood plasma inorganic calcium concentrations of the two groups of heifers, but the control group had a higher (highly significant) mean blood plasma inorganic phosphorus concentration than the vitamin A group. Too much emphasis must not be placed upon this, however, since the control group had a higher mean blood plasma inorganic phosphorus concentration than the vitamin A group at the start of the trial.

CONCLUSIONS

Feeding an average of 40,400 USP units of supplemental vitamin A per day to Holstein heifers receiving a normal ration resulted in no increase in the rate of growth. Increasing the vitamin A supplement to an average of 129,400 USP units per day in addition to the 114,000 USP units supplied daily in the basal ration resulted in a significant increase in body weight gains of Holstein heifers.

Feeding supplemental vitamin A significantly increased the blood plasma vitamin A concentrations and decreased the blood plasma carotene concentrations of Holstein heifers.

Blood plasma inorganic calcium and phosphorus concentrations were not altered by feeding supplemental vitamin A.

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ASSOCIATION ANNOUNCEMENT

COLLEGIATE STUDENTS' INTERNATIONAL CONTEST IN JUDGING DAIRY PRODUCTS

Atlantic City, N. J.—October 25, 1948

Teams from twenty-six State Agricultural Colleges, participated in this, the fourteenth annual contest sponsored by the Dairy Industries Supply Association, Inc., and the American Dairy Science Association.

Following is a list of those who won high standings in the Contest:

ALL PRODUCTS

Individuals

1. Donald R. Moore, Michigan State College
2. Donald H. Pflueger, Iowa State College
3. W. E. Shiffermiller, Ohio State University
4. William H. Hoagland, University of Connecticut
5. Richard E. Lewis, Ohio State University
6. John N. Lewis, Ohio State University
7. Charles H. Fitch, Iowa State College
8. Lester Hankin, University of Connecticut
8. N. V. Kennedy, Mississippi State College
10. Arnold D. Nelson, Iowa State College

Teams

1. Iowa State College
2. Ohio State University
3. Michigan State College
4. Mississippi State College
5. University of Tennessee
6. University of Connecticut
7. University of Maryland
8. University of Massachusetts
9. Pennsylvania State College
10. Cornell University

BUTTER

Individuals

- | | |
|--|-------|
| 1. Donald H. Pflueger, Iowa State College | 4.33 |
| 2. Donald R. Moore, Michigan State College | 8.17 |
| 3. William H. Hoagland, University of Connecticut | 9.00 |
| 4. William C. Flynt, Jr. Mississippi State College | 10.42 |
| 5. Delmer A. Boyce, University of Tennessee | 11.50 |
| 6. Charles H. Fitch, Iowa State College | 11.75 |
| 7. Harold McCracken, University of Tennessee | 11.84 |
| 8. William R. Vial, Purdue University | 12.25 |
| 9. Richard E. Lewis, Ohio State University | 12.67 |
| 10. John N. Lewis, Ohio State University | 13.50 |

Teams

1. Iowa State College	34.58
2. Ohio State University	39.84
3. Mississippi State College	41.92
4. Michigan State College	42.34
5. University of Tennessee	43.51
6. University of Connecticut	44.00
7. Purdue University	50.59
8. University of Nebraska	53.34
9. North Carolina State College	56.25
10. Cornell University	57.25

CHEESE

Individuals

1. William J. Deisley, Pennsylvania State College	28.17
2. John N. Lewis, Ohio State University	28.59
3. Arnold D. Nelson, Iowa State College	29.50
4. Donald R. Moore, Michigan State College	29.75
5. Ralph Whitehead, Michigan State College	31.25
6. Harold A. Newlander, Cornell University	31.34
7. Henry H. Sprowls, Texas Tech.	33.00
8. Donald H. Pflueger, Iowa State College	33.09
9. Robert J. Schutrumpf, University of Maryland	33.43
10. M. V. Kennedy, Mississippi State College	33.50

Teams

1. Michigan State College	94.84
2. Iowa State College	98.09
3. Cornell University	100.50
4. Ohio State University	102.35
5. Texas Tech.	106.85
6. Mississippi State College	110.34
7. University of Tennessee	111.18
8. University of Illinois	112.58
9. University of Connecticut	117.50
10. Purdue University	120.52

ICE CREAM

Individuals

1. Donald H. Pflueger, Iowa State College	29.00
2. John A. McLeod, Jr. North Carolina State College	31.51
3. Ralph Whitehead, Michigan State College	32.00
4. W. E. Shiffermiller, Ohio State University	33.00
5. Donald R. Moore, Michigan State College	33.50
6. George H. Brink, University of Tennessee	34.50
6. Roland I. Zeller, University of Minnesota	34.50
8. Lester Hankin, University of Connecticut	35.00
8. William H. Hoagland, University of Connecticut	35.00
10. Delmer A. Boyce, University of Tennessee	35.50

Teams

1. University of Tennessee	108.50
2. Iowa State College	110.00

3. Ohio State University	111.50
4. University of Minnesota	114.00
5. Pennsylvania State College	116.00
6. Michigan State College	117.00
7. University of Connecticut	118.50
8. University of Nebraska	119.50
9. University of Massachusetts	127.17
10. University of Maryland	129.00

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Individuals

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2. Arnold D. Nelson, Iowa State College	20.45
3. Dan Guyer, Oklahoma A. & M.	22.00
4. Luther O. Meadows, Mississippi State College	23.25
5. William H. Hoagland, University of Connecticut	23.95
5. Donald R. Moore, Michigan State College	23.95
7. W. E. Shiffermiller, Ohio State University	24.35
8. Richard E. Lewis, Ohio State University	25.20
9. Charles D. Spencer, University of Maryland	25.35
9. Alan D. Young, University of Massachusetts	25.35

Teams

1. Iowa State College	76.43
2. Ohio State University	78.45
3. Pennsylvania State College	81.42
4. University of Maryland	85.70
5. University of Massachusetts	86.37
6. Mississippi State College	86.90
7. University of Connecticut	88.65
8. Michigan State College	90.83
9. Oklahoma A. & M.	93.87
10. University of Illinois	94.92

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ABSTRACTS OF LITERATURE

BACTERIOLOGY

241. Biological properties and mouse virulence of *Streptococcus agalactiae* and Lancefield's group "B" streptococci from human sources. A. POMALES-LEBRÓN, P. MORALES-OTERO, AND J. BARALT, School of Tropical Medicine, San Juan, P. R. Proc. Soc. Exptl. Biol. Med., 64, 4: 410-412. April, 1947.

The biological properties and mouse virulence of 70 Lancefield's group B cultures (50 bovine and 20 human) were studied and compared. Aside from the inability to ferment lactose by 8 of the human strains and some difference in the fermentation of glycerol, dextrin, and salicin, the fermentation reactions were very similar for both groups. With the majority of strains the differences between single bovine and human cultures were not greater than those between individual strains from the same source. There was an indication that human strains are more likely to reduce methylene blue than are bovine strains. Strains from human sources possessed a higher mouse virulence than those from bovine sources. R.P.R.

BREEDING

242. Relation between time of fertilization and follicle cell dispersal in rat ova. S. L. LEONARD, P. L. PERLMAN, AND R. KURZROK, Cornell Univ. Proc. Soc. Exptl. Biol. Med. 66, 3: 517-518. Dec., 1947.

Female rats were bred and 12-26 hr. later the ova were removed, examined for the disposition of the follicle cells, and then transferred to a microscope slide to determine if fertilization had occurred. Sixty-five ova, obtained 12-16 hr. post-coitus, were observed to be covered with follicle cells and remained in a compact mass when removed from the oviduct. The ova were similar in appearance to ova recovered from 100 non-bred rats. Spermatozoa were identified within the perivitelline space and polar bodies were present in every instance. Thirty-one fertilized ova, obtained from 10 rats (in 2 instances 13 hr. and in 8 instances 16-26 hr. post-coitus), either were partially or completely denuded of their follicle cells. In not one case were the ova denuded in 12 rats in which hyaluronidase was introduced into both uterine horns during estrus and the ova removed 18-24 hr. later. In all cases, the *in vitro* addition of the uterine fluid of these rats induced complete denudation in 10-20 min. It was concluded that fertilization in the rat ovum occurs before mass displacement of the surrounding follicle cells. R.P.R.

243. Effects of testis hyaluronidase and seminal fluids on the fertilizing capacity of rabbit spermatozoa. M. C. CHANG, Shrewsbury and Tufts Medical College, Boston. Proc. Soc. Exptl. Biol. Med., 66, 1: 51-54. Oct., 1947.

Sixty-five adult non-pregnant rabbits were superovulated by hormonal means and inseminated with a number of spermatozoa needed to fertilize only a small number of ova. Semen of a single male rabbit was collected with an artificial vagina and diluted 1:1,000 with saline. Sperm concentration was determined and saline or sperm added if the concentration of sperm was too high or too low. Then 0.5 ml. of such a suspension was added to each of the following: (a) 0.5 ml. of saline containing a known amount of hyaluronidase, (b) 0.5 ml. of supernatant fluid of normal semen after heating, (c) 0.5 ml. of semen from a vasectomized buck, or (d) 0.5 ml. of saline. One milliliter of these mixtures was inseminated intravaginally. The rabbits were killed 25-30 hr. later, ova flushed from the tubes, and the number of fertilized and unfertilized ova determined. The seminal fluid and not the hyaluronidase was found to have the ability to increase the fertilizing capacity of spermatozoa. R.P.R.

CHEESE

244. Sur l'accident du "Bleu" in fromagerie de pates molles a groute fleurie ("Blue" defect in cheese factories making soft, mold-cured cheese). J. KEILLING, J. CASALIS, JEANNE DUTHEN, L. SIGONNEY, AND IRENE GLASER. Lait, 27, 268: 461-466. Sept.-Oct., 1947.

Blue discolorations on the surfaces of soft cheeses of the Camembert, Coulommiers and Brie types frequently are a serious problem. *Penicillium candidum*, the surface growth commonly present, produces a white, felt-like coat, but growth of *Penicillium glaucum* will result in blue or green spots if sporulation occurs. Observations indicated that bluing did not take place uniformly on both surfaces but was more frequent on the surface which had received prolonged exposure to the anaerobic conditions during drainage. Analyses for ethyl alcohol indicated that this section of the cheese might contain twice to ten times as much as the section not showing the defect, the high alcohol content being the result of yeast growth. Control measures recommended include regular turnings during the draining period and stricter sanitation of draining tables. O.R.I.

245. Étude sur l'eau lié des fromages (Study of the bound water of cheeses). G. Mocquot. Lait, 27, 269-270: 576-595. Nov.-Dec., 1947.

The methods which MacDowall and Dolby developed for determining bound water in Cheddar cheese have been adopted for use on Gruyere curds.

The water-binding behavior of the calcium caseinate fraction, in particular, was studied. Finely cut curds were prepared by coagulating skim milk with rennet at 34° C.; the curds were cooked to 48–50° C. and washed in running water to remove all lactose and other solubles.

Bound water content was determined from a formula based on the extent to which various indicator solutions changed in concentration when the curds were soaked in them for 5 hr. at 10° C. Glycerol, C₆, C₁₂, and C₁₈, sugars, acetone and ethyl alcohol were used as indicator solutions, and bound water content was calculated on the basis of dry matter and on protein content. The values obtained with the sugars were related to their molecular weights, the di- and tri-saccharides yielding values approaching twice and three times as much, respectively, as those for glycerol and the mono-saccharides. Acetone gave values somewhat lower and ethyl alcohol gave values very much lower than the monosaccharides.

The influence of certain variations in cheesemaking methods also was studied. Increasing the degree of pressing and heating the curds decreased bound water content slightly. The addition of salt decreased bound water content greatly as compared to curds receiving no salt. O.R.I.

246. New dehydrated milk products for making soft types of cheese.

W. I. TRETSVEN, Chicago, Ill. *Milk Plant Monthly*, 37, 3: 38–42. 1948. Also, *Milk Dealer*, 37, 7: 44, 45, 106–112. April, 1948.

Cottage cheese production and demand largely is seasonal. This cheese may be stored in brine or in the frozen state for use when skim milk is not so abundant. Manufacture of cottage cheese from dehydrated milk products offers a possibility. Roller-dried nonfat skim milk powders are not suited because the reconstituted solids are quite insoluble and settle out, failing to coagulate during the cheesemaking operation. Spray-dried nonfat milk solids may be used in manufacturing cheese, but their use is somewhat limited because the coagulated reconstituted milk may not shrink adequately to yield a good quality cheese. Several variations in reconstituting the nonfat dry milk solids for cottage cheese making may be employed. New products are on the market which, when added to the reconstituted milk, aid materially in cottage cheese manufacture. The use of Lacal results in an increased yield of cheese and a reduction in losses in the whey; whey disposal problems are alleviated. While developed primarily for manufacturing cottage cheese, Lacal has other possible uses, such as, for carrying mother culture and starters; for making Baker's cheese, which drains readily without being sticky or pasty; for manufacturing Newfchatel and cream cheese; and for emulsifying dry milk fat into the reconstituted product for creaming cottage cheese. G.M.T.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

247. Dry milkfat as a form of storage fat in the dairy industry. R. J. REMALEY, Kraft Foods Co., Chicago, Ill. *Milk Plant Monthly*, 37, 3: 43, 57. 1948.

Dry milkfat as opposed to butter oil is produced from fresh cream. The cream is heated to a temperature ranging from 170–190° F. and separated into 80% fat. By running the plastic cream through a homogenizer into a continuous type settling tank, a product containing approximately 98% fat and 2% moisture is secured. By centrifuging, a dehydrated milkfat is obtained. The standard of the U. S. Army Quartermaster Corps requires not less than 99.8% milkfat in the final product. Such milkfat may be stored at 40° F. for a period of at least 6 months with little analytical or organoleptic change. Dry milkfat may be used in any place that butter can be used. In areas lacking proper refrigeration and where there is a shortage of milk supply, a combination of nonfat dry milk solids and dry milkfat makes possible most types of dairy products. Dry milkfat should not be confused with butter oil. G.M.T.

248. Sur la synérèse des caillés lactiques obtenus par l'action de *Streptococcus thermophilus* (On the syneresis of lactic curds as a result of the action of *Streptococcus thermophilus*). J. KEILLING, J. CASALIS, AND IRENE GLASER. *Lait*, 27, 268: 449–461. Sept.–Oct., 1947.

In the manufacture of yoghurt and similar products, the separation of whey is undesirable. A mixture of *Streptococcus thermophilus* and *Thermobacterium yoghurt* is used in making yoghurt. The authors have investigated the effects of such factors as rates of heating, pasteurization temperature, length of holding time, and rate of cooling upon the degree of whey separation in cultures of *S. thermophilus* incubated at 45° C. Whey separation was at a minimum where the milk was heated either rapidly or slowly to temperatures above 84° C. with no holding period. At 80° C. whey separation was negligible with a holding time of 10 min. or longer. O.R.I.

DISEASES

249. Bovine brucellosis—A problem for the whole dairy industry. G. R. SPENCER, Dept. of Vet. Med., Univ. of Wis., Madison. *Milk Plant Monthly*, 37, 5: 42–46. 1948.

Brucellosis, formerly known as Bang's disease or contagious abortion, is responsible for sterility and lower production of milk, both of which are of great economic importance to the dairy industry. Pasteurization of milk

will kill *Brucella* organisms and is a positive protection to consumers. Nevertheless, precautions must be taken to protect animals from infection with the organisms. The bacteria causing the disease are taken into the body with food or water. Animals infected with *Brucella* may recover yet remain as carriers, spreading the infection throughout the herd. Nation-wide attention is given toward control. Three methods of brucellosis control are in use at the present time, namely: (a) test and slaughter, (b) use of quarantine, sanitation and restriction of the movement of infected cattle, and (c) vaccination with a live culture of *Brucella* called *Strain 19* to produce resistance to the disease. An agglutination test known as the "ring" or Fleisohauer test promises unusual possibility in detecting brucellosis-infected milk at the receiving platform. Research is underway, particularly by Dr. I. F. Huddleson, Michigan State College, to develop better vaccines than are available at the present time. The success in virtual elimination of bovine tuberculosis from the dairy cows offers an incentive to the possibility of eradication of brucellosis from the dairy herds. G.M.T.

FEEDS AND FEEDING

250. Essai d'augmentation de la matiere grasse du lait par l'administration de vitamines du groupe B contenues dans la levure de biere (Attempt to increase milk fat by the administration of the B group vitamins in brewer's yeast). D. GIANNOTTI, Pisa University. Lait, 27, 269-270: 561-576. Nov.-Dec., 1947.

Brewer's yeast at rates of 5, 10 and 20 g. per day was fed in a paired feeding and production test over a 2.5-month period. The results indicated that neither fat content of the milk nor milk production was augmented by these additions to the ration. O.R.I.

251. Feeding urea to dairy cows with special reference to the palatability of feed mixtures containing urea. J. E. BOWSTEAD AND H. T. FREDEEN, Univ. of Alberta, Edmonton. Sci. Agr., 28, 2: 66-78. Feb., 1948.

Results of practical feeding trials carried out over a number of years in the use of urea as a protein substitute in dairy cattle feeding are reported. Attempts to compare the value of urea at a level of 2% in a ration resulted in failure due to lack of palatability. Cows suddenly switched to a ration containing 2% urea and those gradually introduced to such a ration consumed their grain allowance without relish and after 12 weeks developed an aversion to rations containing urea. The inclusion of corn made the ration somewhat more palatable, and molasses increased palatability considerably. The authors suggest that this aversion is not due to taste but to the possibility that the bacterial flora of the rumen is altered when urea is fed.

Individual cows vary greatly in their tolerance for urea, although a grain ration containing 0.5% is eaten fairly readily. The addition of cobalt possibly may be an aid in the feeding of urea. O.R.I.

252. Digestibility studies with ruminants. XI. The effect of the nutritive ratio of a ration upon its digestibility by cattle. C. J. WATSON, J. W. KENNEDY, W. M. DAVIDSON, C. H. ROBINSON, AND G. W. MUIR, Central Experimental Farm, Ottawa, Canada. *Sci. Agr.*, 27, 12: 600-608. Dec., 1947.

Using timothy hay of good quality, barley, and soybean oil meal, mixed rations were made up with nutritive ratios varying from 1:2.63-1:8.41. Eight rations were used in the experiment. The coefficients of digestibility of these rations were determined with 8 grade Shorthorn steers. The coefficients of digestibility of the individual feeds also were determined, and these were used to calculate the coefficients of digestibility of the mixed rations. By comparing the coefficients so calculated with those obtained in the digestion trials, it was shown that the nutritive ratios of the mixed rations did not influence their digestibility. O.R.I.

253. Epithelial keratinization as evidence of fetal vitamin A deficiency. J. G. WILSON AND J. WARKANY, Univ. of Rochester and Univ. of Cincinnati. *Proc. Soc. Exptl. Biol. Med.*, 64, 4: 419-422. April, 1947.

Keratinizing metaplasia was found in epithelia of the genito-urinary tract of fetal rats taken from mothers that were deficient in vitamin A during pregnancy. Prior to the 18th day of gestation, stratified, keratinized epithelium was not found; however, all fetuses older than 18 days showed keratinizing metaplasia in at least some of the genito-urinary epithelia. By the 20th day keratinization was seen in the dorsal wall of all organs distal to the termination of the genital ducts. Epithelia in other parts of the body were not affected. R.P.R.

254. The stability of iodine in iodized rock salt. W. M. DAVIDSON AND C. J. WATSON, Central Experimental Farm, Ottawa, Canada. *Sci. Agr.*, 28, 1: 1-5. Jan., 1948.

Blocks of sodium chloride containing added potassium iodide at the rate of 1 oz. per 5 lb. of salt were put up by a commercial manufacturer. Protective agents such as calcium stearate and sodium thiosulfate were added to some mixtures and ferric oxide was added to others. Granulated salt representative of each mixture also was stored in glass jars.

Blocks of each of these mixtures were exposed to summer pasture conditions for 2 months, to conditions simulating stall feeding for 16 months, and to storage in cartons at barn temperatures for 9 months. The granulated salt was examined after 3 and 13 months.

Under summer pasture conditions complete disappearance of the iodine took place in 2 months from the blocks, even when protective agents were present. Under stall conditions the iodine showed fair stability for 2 months, but only small amounts remained after 17 months. In cardboard cartons there was no loss of iodine over a 9-month period, and iodine in the granulated salt remained stable for 15 months.

O.R.I.

255. Method of controlling the growth and fattening rate of livestock and poultry and composition used in connection therewith. C. W. TURNER AND E. P. REINEKE. (Assigned to American Dairies, Inc., and Quaker Oats Co.) U. S. Patent 2,438,353, March 23, 1948 (13 claims). Official Gaz. U. S. Pat. Office, 608, 4: 769. 1948.

Livestock and poultry may be grown and fattened at an increased rate, without affecting milk or egg production, by feeding during a selected span of life, thyroprotein (example: iodinated casein) which stimulates metabolism and then feeding substances containing the thioureyline radical (example: thiouracil) in the range of 0.01 to 0.15% for each 100 lb. of feed to arrest thyroid activity.

R.W.

FOOD VALUE OF DAIRY PRODUCTS

256. The nutritive value of commercial ice cream. A. C. DAHLBERG AND J. K. LOOSLI, Dept. of Dairy Industry and Dept. of Animal Husbandry, Cornell Univ., Ithaca, N. Y. Ice Cream Trade J., 44, 4: 56, 99-101. April, 1948.

Three batches of commercial ice cream were made, the composition being as follows: fat, 12%; nonfat milk solids, 10%; sugars, 15.5%; gelatin, 0.3%; egg solids, 0.2%; and total food solids, 38%. The ice cream was found to contain the following nutrients per 100 g. of ice cream: calories, 206; protein, 3.85 g.; fat, 12.06 g.; carbohydrate, 21.31 g.; total minerals, 0.81 g.; calcium, 0.112 g.; phosphorus, 0.105 g.; iron, 0.120 mg.; thiamine, 0.038 mg.; riboflavin, 0.236 mg.; niacin, 0.098 mg.; vitamin A, 548 I.U.; and ascorbic acid, 0 mg.

W.H.M.

257. Calcium enriched meat compared with milk as source of calcium, phosphorus and protein. I. MCQUARRIE, MILDRED ZIEGLER, AND I. H. MOORE, Univ. of Minn. Medical School. Proc. Soc. Exptl. Biol. Med., 65, 1: 120-121. May, 1947.

From the viewpoint of Ca, P and protein, the nutritional value of a Ca-enriched meat diet was compared experimentally with a milk diet containing the same quantities of essential food constituents. Balance studies were carried out in 2 young male patients. Total carcass and separate femur

analyses were made on rats which were on 2 types of diet. The results indicated that the Ca-enriched meat diet was equally as good as the milk diet as a source of Ca, P and protein. R.P.R.

258. Dental caries in the cotton rat. IX. Effect of milk rations. E. P. ANDERSON, J. K. SMITH, C. A. ELVEHJEM, AND P. H. PHILLIPS, Univ. of Wisconsin. Proc. Soc. Exptl. Biol. Med., 66, 1: 67-69. Oct., 1947.

Cotton rats were placed on experiment at weaning (20-25 g.). Rats from each litter were distributed as equally as possible between the control and experimental groups. Each experiment lasted for 14 weeks, during which time body weights were recorded at biweekly intervals. At the end of the experiment the animals were killed and the incidence and extent of carious lesions estimated. Milk was found to be protective against dental caries, and zero scores in rats fed only liquid milk were obtained. Animals that received milk to which sucrose, glucose, or dextri-maltose (sugars that produce caries when fed in dry rations) had been added exhibited low caries scores as compared with controls on a cariogenic ration. The indices of dental caries in rats receiving approximately one-third of their caloric intake as liquid milk and the remainder as a cariogenic ration were less by 50% than those of litter-mate controls not receiving milk. R.P.R.

259. Fractionation of lacteal liquids. F. K. DANIEL. (Assigned to Sun Chemical Co.) U. S. Patent 2,437,080, March 2, 1948 (5 claims). Official Gaz. U. S. Pat. Office, 608, 1: 154. 1948.

Cold milk, skim milk, whey, etc., may be continuously fractionated into 2 fractions by an osmotic diffusion and stratification dialysis procedure, employing a series of cells separated by cellophane or other semi-permeable membrane. The proteinaceous fraction, consisting of undenatured casein, lactalbumin, and lactoglobulin, is suitable for the diet of those suffering from pancreatin deficiency or diabetes, for use in ice cream, as a whipping aid, emulsifying agent, etc. The diffusate, consisting of the lactose, ash, water and soluble vitamins, is suitable as a culture medium for micro-organisms and as a source of vitamins and other compounds. R.W.

ICE CREAM

260. Selling through franchise dealers. E. THOM. Ice Cream Rev., 31, 9: 40, 41, 88, 90. April, 1948.

The program of the Bridgeman Creameries for building sales of ice cream through a selected group of 22 franchise dealers in Minneapolis and St. Paul is discussed. Franchise dealers are required to sign a contract

which will insure full compliance with Bridgeman's merchandising program. The equipment to be installed and its arrangement, the training of fountain personnel, advertising and promotional activities, the sanitary standards to be maintained, and the items to be sold or featured all are controlled by the company. The dealer, in turn, is assured a satisfactory profit on his fountain operation. Factors which tend to attract trade to the stores are: Customers receive full value for their money; a rule at all Bridgeman stores is generous servings reasonably priced. The quality of ice cream is as high as any in the market and the product is maintained under ideal storage conditions. The store surroundings are conducive to the greater consumption of ice cream. Customers are reminded of ice cream whenever they walk into a Bridgeman store. Ice cream is the featured item at all stores. The dealer actively promotes the sale of ice cream.

W.J.C.

261. The retail store, past—present—future. Part 3. D. GHORMLEY. Ice Cream Trade J., 44, 4: 48-49, 88-92. April, 1948.

Findings of the Stanford University survey show a marked increase in the number of retail ice cream stores since 1946. The average gross sales per store in 1946 were \$5,250 per month compared to \$4,190 per month in 1940. The annual sales volume per store reporting was 24,225 gallons.

Forty-one per cent of the sales of such stores are to minors, 45% to persons between 21 and 50 yr. of age, and 14% to persons over 50 yr. of age. Seventy-four per cent of purchases are made by women. Fifty per cent of specialized retail ice cream stores are located in suburban business districts. Fifty-eight per cent of customers drive to stores, indicating the value of parking lot facilities. Ninety per cent of store operators favor parking lots on the premises. Fifty-five per cent of the firms reporting use training manuals for help. Employees are 64% women, 20% men and 16% minors. Sideline products have come to be accepted in virtually all stores. W.H.M.

262. Consumer buying habits on ice cream. ANONYMOUS. Ice Cream Trade J., 44, 4: 44-45, 103, 104. April, 1948.

The Chicago Tribune has made a survey by telephone of 271 families in Chicago to determine when, where and by whom ice cream purchases are made. More than 50% of the purchases were made on Friday and Saturday. In 53.1% of the cases the ice cream purchased was served at a meal, usually at the evening meal. The heaviest buying period was from 3 to 9 p.m., with the peak at 4 to 7 p.m. The housewife made 45.1% of the purchases, the husband 19.9% and the children made the remaining purchases. When the husband made the purchases, in two-thirds of the cases he did so after he returned home from work. The two chief types of stores where pur-

chases were made were the drug store in 31.4% and the grocery store in 27.7% of the cases. Convenience was given as the main reason for making the purchase at a particular type of store, followed by brand preference in about one-third of the cases.

A large portion of the ice cream going into the home still is not a part of the daily menu but rather is being used as a mid-afternoon snack or as a refreshment late in the evening. With convenience cited as the main reason for the purchase at a particular outlet, the grocery store in time may be the major outlet.

W.H.M.

263. Sensible pricing at the ice cream soda fountain. ANONYMOUS. *Ice Cream Rev.*, 31, 9: 44-45. April, 1948.

Reduction in ice cream gallonage in recent months is attributed in part to the practice of over-pricing soda fountain items by some dealers. It is not uncommon for the cost of ice cream to the consumer to advance well over \$1.00 per gallon at the soda fountain when the wholesale price of ice cream advances by only 15 cents per gallon. Such practice tends to price the soda fountain out of business. An educational program with dealers is suggested as the best method to combat this practice. Supplying dealers with concrete cost information, showing exact cost figures for individual servings of ice cream, and toppings at varying prices per gallon for ice cream and toppings have proved very successful in holding soda fountain price structures in line, according to the reports of one company. Two charts are reproduced in the article and should prove valuable to anyone interested in discussing fair price structures at soda fountains with their dealers. A 45% gross profit is considered ideal for soda fountain operation, with the desirable range from 40 to 50%.

W.J.C.

MILK

264. Continuous flow pasteurizer. H. I. SOUTHERWICK. (Assigned to the Do All Co.) U. S. Patent 2,438,582, March 30, 1948 (4 claims). *Official Gaz. U. S. Pat. Office*, 608, 5: 949. 1948.

In a simple sanitary continuous pasteurizer for such fluids as milk, the heating is accomplished by the resistance offered by the milk to an electric current as it flows between two electrodes. The electrodes are cooled by the incoming cold milk. Close temperature control is maintained by altering the rate of flow of the milk by means of a valve which is actuated by the magnitude of the current flowing through the liquid. This arrangement automatically compensates for any fluctuation in the current supply and changes in the resistance of the milk due to temperature.

R.W.

265. Production of recombined and reconstituted milk. O. E. STAMBERG, Industrial Research Laboratories, Milwaukee, Wis. Milk Dealer, 37, 7: 47, 118-120. April, 1948.

The use of reconstituted whole milk, a product made by properly dispersing dry whole milk powder in the required amount of water (the term sometimes is employed for a product made from evaporated milk and water) and of recombined whole milk, a product made by properly combining non-fat dry milk solids with cream, butter or milk oil and the required water, is discussed. These products all are nutritionally excellent. Reconstituted milk produced from evaporated milk has a pronounced cooked flavor which detracts from its acceptance for drinking purposes. Whole milk powder must be used quite fresh; otherwise the milk will have off-flavors, referred to generally as "chalky", "oxidized", or "tallow". The production of recombined milk requires more technological knowledge than the reconstitution of whole milk powder, but it generally is conceded that recombined milk is superior in quality to most products from whole milk powder.

C.J.B.

266. Promoting greater milk production. M. H. BRIGHTMAN. Milk Dealer, 37, 7: 42, 43, 98-100. April, 1948.

Charts are presented and discussed showing that prices of dairy products historically have kept ahead of the prices of farm products other than dairy; income from dairy cattle remains more consistent, and usually higher, than that from either food or feed grains; and milk production is not keeping up with the human population of the United States. The feed situation is more optimistic than it was a few months ago. The author concludes that the dairy business is a good business, that it has returned more money in the past and probably will return more in the future than most other types of farming, that cows that are capable of producing milk efficiently should be kept, that good heifer calves should be raised, and that we should feed efficiently to get maximum milk production.

C.J.B.

267. Milk bottle utility device. J. STRANSKY. U. S. Patent 2,438,024, March 16, 1948 (5 claims). Official Gaz. U. S. Pat. Office, 608, 3: 600. 1948.

A device for attaching to the top of a standard milk bottle is described which provides the following features: (a) a tube inserted through the disc cap which allows pouring of the milk without removing the cap and exposing the product to the exterior lip of the bottle, and (b) a receptacle for attaching notations to the person delivering the milk or for holding money for payment of the milk.

R.W.

PHYSIOLOGY

268. Effects of gonadotrophic hormones on lactation. G. M. C. MASSON, Univ. of Montreal. *Proc. Soc. Exptl. Biol. Med.*, 66, 3: 506-508. Dec., 1947.

Forty-one lactating albino rats weighing 250-300 g. were divided into 6 groups, 5 of the groups receiving a gonadotrophin of either pituitary or chorionic origin. Pregnant mare serum had a strong inhibitory effect on lactation; only 18% of the litters survived until the 16th day. The gonadotrophic principle of the urine of pregnant women produced a slight inhibition of lactation, while an anterior pituitary preparation was inactive. Histological studies of the ovary, vagina and uterus indicated that the administration of pregnant mare serum resulted in a high level of estrogen and progesterone. R.P.R.

269. Effect of sex hormones on pituitary lactogen and crop glands of common pigeons. J. MEITES AND C. W. TURNER, Dept. of Dairy Husb., Univ. of Mo., Columbia. *Proc. Soc. Exptl. Biol. Med.*, 64, 4: 465-468. April, 1947.

Groups of 16 to 20 mature pigeons of both sexes were injected subcutaneously daily for 10 days with either estrone, diethylstilbestrol, progesterone or testosterone propionate. The pigeons were killed on the day after the last injection, weighed and examined under light for evidence of visual proliferation. The pituitaries of each group were removed, weighed, and assayed for their lactogen content by injecting them intradermally over the crop glands of 10 common pigeons. The results were all negative, and it was concluded that the pigeon pituitary, unlike the mammalian, is refractory to the administration of gonadal hormones. R.P.R.

270. Effect of thiouracil and estrogen on lactogenic hormone and weight pituitaries of rats. J. MEITES AND C. W. TURNER, Dept. of Dairy Husb., Univ. of Mo., Columbia. *Proc. Soc. Exptl. Biol. Med.*, 64, 4: 488-492. April, 1947.

The feeding of a ration containing 0.1% thiouracil for 24 days to young female rats reduced the lactogen content of the pituitaries below that in normal rats. In rats fed thiouracil for 14 days, the subsequent administration of thiouracil and 100 I.U. of estrone daily for 10 days failed to maintain the normal level of the lactogenic hormone. However, there was an increase in the lactogen content of pituitaries of rats that received estrone and thiouracil daily for 21 days. Pituitary weight was increased by the administration of thiouracil as well as thiouracil plus estrogen. Thyroid hypertrophy was less in rats receiving thiouracil plus estrogen than it was in rats receiving thiouracil alone. R.P.R.

SANITATION AND CLEANSING

271. Some comparisons of the disinfecting properties of hypochlorites and quaternary ammonium compounds. L. SHERE, The Diversey Corp., Chicago, Ill. *Milk Plant Monthly*, 37, 3: 66-69. 1948.

A considerable variation in the germicidal effect of different quaternary compounds is reported. Increasing water hardness has a great and variable effect in reducing the germicidal power of different quaternary compounds. Anionic wetting agents commonly used in cleaning compounds affect adversely the germicidal action of quaternary compounds. Milk solids adversely affect the germicidal action of quaternary compounds and hypochlorite. However, with milk solids present, hypochlorite kills at a lower concentration than any of the quaternaries tested. Lowering the water temperature below 68° F. reduces the disinfecting power of quaternary compounds. Precipitation formed in hard water by the water softening action of certain alkalies absorbs the quaternary compounds and removes them from solution; these precipitates do not absorb hypochlorites. Increasing water hardness, addition of anionic wetting agent, and reduction of water temperatures below 68° F. have no adverse effect on the disinfecting action of sodium hypochlorite.

G.M.T.

272. Good housekeeping in the dairy plant. J. H. ERB, The Borden Co., Columbus, Ohio. *Milk Plant Monthly*, 37, 4: 70-71. 1948.

Good housekeeping, beginning with a building in good repair, results from cooperative effort between the manager and the plant organization. A special housekeeping committee which makes regular monthly inspections and written reports in each plant is most helpful. Forms aid in calling attention to items which should be judged and inspected. Special items in plant housekeeping and operation which may reflect good housekeeping are: fit of valves, broken glass on floor, accumulation on sills, lack of paint, lack of dairy sanitation, excess grease on equipment, equipment not polished, untidy laboratory tables, dirty window panes, cleanliness of halls, condition of locker room, employee uniforms, freedom from insects and rodents, orderliness of stock room, and neatness and appearance of platforms. Dairy plants have made great progress in good housekeeping but much improvement still is needed.

G.M.T.

273. How to get cans clean. V. SCHWARZKOPF, Lathrop-Paulson Co., Chicago, Ill. *Milk Plant Monthly*, 37, 3: 46-51. 1948.

Before the milk can can be cleaned, one must have an understanding as to what constitutes a clean can. Such cans must look, feel and smell clean, be dry and be practically too hot to handle when released from the can

washer. Keeping the machine clean, maintaining optimum temperature, maintaining proper strength solutions, and keeping the machine in good operating condition will influence good can washing. The author summarizes the solution to the problem of getting cans clean as follows: Make someone responsible for the proper operation and care of the can washer, and see that this responsibility is fully assumed. Keep machine clean, well groomed and lubricated. Use temperatures for all treatments which will provide maximum cleaning, maximum destruction of bacteria and maximum dryness without lime or scale deposit. Never reach or exceed the critical temperature of the cleaning compound being used. Maintain proper strength solution for washing and rinsing to provide maximum cleaning without formation of lime or scale. Use relatively clean water for washing all cans. Select the cleaning compound most suitable for the can washer being used. Do not alternately use alkaline and acid cleaners if the temperatures used reach or exceed the critical temperature of the alkaline cleaner.

G.M.T.

MISCELLANEOUS

274. Statistical evaluation of growth curves. O. L. DAVIES, Imperial Chemical Industries, Manchester, England. *Proc. Soc. Exptl. Biol. Med.*, 66, 3: 567-568. Dec., 1947.

The comparison of growth curves by the method proposed by Weil is an oversimplified method of analysis and not a valid one. The *t*-test is a valid method, provided the apportionment of the animals between groups has been carried out in a strictly random manner. Some improvement probably would result when the final weights are corrected for the variations in the initial weight by the covariance method discussed by Fisher. A more complete method is to fit regression lines, by procedures such as the method of least squares, to the growth curve of each animal, using, if required, a simple transformation to the time and weight scale in order to produce a simple curve.

R.P.R.

275. Statistical evaluation of growth curves. C. S. WEIL, Mellon Institute, Pittsburgh, Pa. *Proc. Soc. Exptl. Biol. Med.*, 64, 4: 468-470. April, 1947.

Weight data were expressed in the form of frequency distributions, each weighing being considered a point in the distribution. The method of chi square revealed similar probability levels of significance, as did the ratio of 3 times the standard error of the difference, to the difference between the mean weights. The calculation of the coefficient of skewness gave values that justified the use of normal statistics. The use of the chi square test on the distributions was deemed the method of preference for showing differences in time-weight data.

R.P.R.

276. Radioautographs in which the tissue is mounted directly on the photographic plate. T. C. EVANS, Columbia Univ. Proc. Soc. Exptl. Biol. Med., 64, 3: 313-315. March, 1947.

Tissue sections containing radioactive material were mounted directly on photographic emulsion in the dark room. After suitable exposure, the plate was developed and the tissue stained with Harris' hematoxylin and aqueous eosin, then dehydrated, cleared, and mounted in permount or balsam. The preparation was studied microscopically as the autographic image was in place just below the tissue. R.P.R.

277. Traffic cops of the pipelines. H. J. BARTLETT, Crane Co. Milk Dealer, 37, 7: 52-60. April, 1948.

Check valves are designed to permit flow in one direction only; they close automatically if flow reverses. There are only two basic types, i.e., swing check valves and lift check valves. Disregarding exceptions, it can be assumed that the greatest field of usefulness for swing check valves is in water and other liquid service. Lift check valves definitely are considered to be superior to swing check valves for service on steam, air, gas, and vapors in general. Different designs of each type are described. C.J.B.

278. This business of advertising. R. E. SHANNON, The Evening Journal, Washington, Iowa. Milk Dealer, 37, 7: 158-159. April, 1948.

Advertising is discussed under the headings of sell personality, local advertising methods, and advertising suggestions. Know your audience; understand that they are more interested in themselves and their needs than they are in you. Prepare your advertising carefully; plan it well in advance and humanize it by using names and local references. Do not use heavy descriptions, waste words and space in claims of superiority, or try to educate the public in the technical phases of your business. C.J.B.

ABSTRACTS OF LITERATURE

BOOK REVIEW

279. Dairy bacteriology. B. W. HAMMER. 3rd edn. 593 pp. \$6.00.
John Wiley and Sons, Inc. 1948.

This new edition of the standard American book in this field reflects the increased amount of available material by including 111 pages more than found in the preceding edition, and by including material from a large number of recent publications. The chapter on "Tests for the General Quality of Raw Milk" has been moved almost to the front of the book, the chapter on milk-born diseases has been made considerably more concise and a chapter on "Bacteriology of Dairy-Plant Water Supplies" has been added, to mention only a few of the major changes. An increased number of illustrations and a greater tendency to point out the practical applications of the various test and control procedures help to make the book of greater value to both student and processor. The book is well-indexed and is printed and bound very satisfactorily. This new edition should be in the hands of all who are concerned with the technical aspects of the handling of dairy products, and many others could profit very considerably by greater familiarity with the material presented.

F. E. Nelson

BACTERIOLOGY

280. A comparative study of commonly used staining procedures for the direct microscopic examination of milk. B. S. LEVINE AND L. A. BLACK, U. S. Public Health Service, Cincinnati, Ohio. *J. Milk and Food Technol.*, 11, 3: 139-148. May-June, 1948.

An aqueous methylene blue dye is readily incorporated in the milk proteins and may cause frequent overstaining. Strong contrasts are attained at the expense of delicate color shades, resulting in the loss of visibility of bacteria whose affinity for the dyes is comparable to the milk proteins forming the background of the smear. Sulfuric acid causes a distortion of the cells. Hydrochloric acid appears to cause a denaturation of the milk proteins, resulting in loss of adhesive properties. All the acids studied, including acetic, cause a light background of the stained smear, thus making lightly stained bacteria imperceptible to the eye. Methylene blue and basic fuchsin solutions require a high acidification, resulting in many disadvantages, while the red background is fatiguing to the eye. On the basis of these studies the authors believe that procedures for staining milk smears can be improved.

H. H. Weiser

281. Common micro-organisms in defective milk products and their control. R. V. HUSSONG AND W. O. NELSON. *Can. Dairy Ice Cream J.*, 26, 10: 28-30. Oct., 1947.

The article gives the methods of staining, the types of bacteria found in dairy products and the microscopic appearance of these micro-organisms.

H. Pyenson

282. Recent developments in milk quality control. A. R. M. MACLEAN. *Can. Dairy Ice Cream J.*, 27, 5: 31. May, 1948.

The most reliable test for the pasteurizability of milk is that of laboratory pasteurization. A plate count of 50,000 colonies per cc. is regarded as plant pasteurizable. For practical control, the plate count should be regarded as an index rather than a standard for the quality of pasteurized products.

H. Pyenson

283. Microlysine-tear gas preserves milk. N. E. GIBBONS AND HELEN J. BROWN. *Can. Dairy Ice Cream J.*, 27, 5: 36-37. May, 1948.

Microlysine is a pure form of trichloronitro-methane for use in food products. It is better known as chloropicrin, one of the tear gases. Microlysine was used during the war in France to preserve milk. At all concentrations used, the numbers of viable organisms were greatly reduced. The reduction was roughly proportional to the concentration of microlysine, and the organisms growing at 98° F. were affected more than those growing at 70° F. No information is available about its effect on pathogens. Its use in milk is prohibited in Canada.

H. Pyenson

284. Possible uses of ultraviolet radiation in the dairy industry. E. I. MORWICK. *Can. Dairy Ice Cream J.*, 27, 3: 34-36. March, 1948.

A new and practical method of controlling mold, bacteria and other micro-organisms is through the use of selected ultraviolet radiation. In the dairy industry sterilamps can be used in the cow barn to reduce the number of bacteria in the air and thus reduce the number of bacteria in the milk. Other uses are for the milk room, milk cans, and in the distributors plants over the bottle filler, conveyors and in those cases where milk is exposed to the air.

H. Pyenson

BUTTER

285. Studies on the Fritz butter machine. J. A. PEARCE. *Can. Dairy Ice Cream J.*, 27, 4: 48-49. April, 1948.

Factors with no effect on production are: (a) the use of pasteurized or unpasteurized cream; (b) the use of a summer and winter cream; (c) the

use of cream with various acidities; (d) decreasing the number of paddles in the churning chamber; (e) allowing the butter granules longer passage in the butter press; (f) 16 alterations in the kneading chamber; and (g) width of the openings in the variables gates. Factors affecting butterfat loss were anti-clockwise rotation of the paddles and low paddle speed. Factors affecting production were increased fat content, cream temperature, the position of the valve in the constant level tank, rotation of the paddles, speed of the paddle, and jacket temperature. Factors affecting moisture content are cream temperature, the position of the valve opening, rotation of the paddles, jacket temperature, the temperature of the butter granules, and the auger speed. The Fritz butter contained more air than the sample of churn butter.

H. Pyenson

286. Report on the preparation of butter samples. H. J. MEURON, Food and Drug Administration, San Francisco, Calif. J. Assoc. Offic. Agr. Chemists, 31, 2: 318-327. 1948.

Collaborative results of a study of methods for the preparation of butter samples for analysis are presented. The following method was adopted as official: Soften the sample by warming to 39° C., shaking intermittently to reincorporate any separated fat. When optimum fluidity is attained, shake vigorously at frequent intervals until the sample cools to a homogeneous semi-liquid of the consistency of thick cream. Optimum fluidity is described as that point where the emulsion is still substantially intact but the mixture moves freely on shaking. Weigh portion for analysis promptly.

F. J. Babel

287. Fat losses in creamery operation. C. G. OBEE. Can. Dairy Ice Cream J., 27, 2: 27-28. Feb., 1948.

Since the butter industry is working on a small margin of profit, the losses must be kept down to a minimum. It is important to prevent and minimize losses in composition; errors in weighing and testing cream; losses in cans and weigh cans; cream used for samples; improper rinsing and draining of vats, pumps, and pipes; spills from cans, vats and churns; and losses with some mechanical printers.

H. Pyenson

CHEESE

288. Pasteurization of milk for cheesemaking. R. W. BROWN. Can. Dairy Ice Cream J., 26, 10: 26-27. Oct., 1947.

The quality of the cheese as indicated by the scores for flavor is definitely in favor of the pasteurized milk cheese. There were 888 flavor scorings with 344 comparisons made on 296 samples of cheese, half of

which were made from raw milk and half from pasteurized milk. On the basis of these comparisons, the cheese made from pasteurized milk showed an average betterment in the flavor score of 1.198 points, or a value that frequently meant the difference between first and second grade cheese.

H. Pyenson

289. How to increase yield in cheesemaking. E. C. DAMROW. Can. Dairy Ice Cream J., 27, 2: 50. Feb., 1948.

To increase yield in cheesemaking refuse off-flavor milk at the intake, cut curd slightly on the soft side, use a metal plate to shove off curd sticking on the sides of the vat and use mechanical agitating instead of rake stirring. Mechanical agitation will increase yield about 25 lb. of cheese per 10,000 lb. of milk over rake stirring.

H. Pyenson

290. Correcting defects in Canadian cheese. J. M. BAIN. Can. Dairy Ice Cream J., 27, 5: 58. May, 1948.

The main defects encountered in Canadian cheese are fruity, not clean, openness, rancid and extraneous matter. To overcome these defects cheese factories need better light, ventilation, sanitary equipment and closed-off boiler rooms.

H. Pyenson

291. Studies on openness in Cheddar cheese. E. G. HOOD AND C. A. GIBSON. Can. Dairy Ice Cream J., 27, 3: 31-33. March, 1948.

When 1 hr. or longer was allowed between milling and salting, 100% of the experimental cheese were graded as close. When less time was allowed between milling and salting, 89.5% of the cheese were faulted to some degree for openness.

H. Pyenson

292. Improving starters to make uniform cheese. E. C. DAMROW. Can. Dairy Ice Cream J., 26, 10: 58. Oct., 1947.

For a good starter and uniform cheese, the following simple rules should be followed: (a) clean and sterilize starter equipment thoroughly with clean water in a clean wash sink; (b) use this equipment, thermometers, pails and containers for starter handling only; (c) eliminate off-odors from floors or sewer odors in plant; (d) do not let your breath come in contact with the starter or mother starter; and (e) get a fresh starter weekly from a reliable source.

H. Pyenson

293. Extraneous matter in Canadian Cheddar cheese. R. THIBODEAU. Can. Dairy Ice Cream J., 26, 10: 31-33. Oct., 1947.

A new modification of the citrate method of detecting extraneous matter in cheese has been developed using a smaller sample of the size pro-

duced by a trier and may be designated as a "micro-test." The filter area also has been reduced in proportion to the reduction in size of the sample. Since the method is quick, it can be used for a wide-spread educational program to control extraneous matter, or for a system of grading cheese according to the amount of foreign matter it contains, just as butter is graded according to the amount of salt it contains. H. Pyenson

294. Control of extraneous matter in Cheddar cheese. J. P. JULIEN, R. DUMAIS, AND R. THIBODEAU. *Can. Dairy Ice Cream J.*, 26, 10: 34-36. Oct., 1947.

When the "micro-test" for sediment is used, it is possible to exercise control over extraneous matter in cheese on a large scale. A new method of preservation of cheese samples is described. It consists of keeping the samples in a sealed jar containing chloroform vapors. Chloroform proved to be an ideal preservative for this type of work, the samples remaining completely soluble and showing no signs of mold during long periods of time. H. Pyenson

295. Dichlorethyl ether in the control of cheese mites. W. S. McLEOD AND R. W. BROWN. *Can. Dairy Ice Cream J.*, 27, 3: 80. March, 1948.

All cheese was removed from the room and dichlorethyl ether was applied at the rate of 1 lb. per 1000 cubic ft. The room was then closed and locked. On the following day the cheeses were washed and returned to the curing-room. It is advised that a respirator be worn while performing the fumigation. All mites were killed by a single application of dichlorethyl ether, and no reinfestation occurred during a period of 8 months. H. Pyenson

296. Retention of certain minerals and water-soluble vitamins in cheesemaking. O. R. IRVINE, L. R. BRYANT, W. H. SPROULE, E. V. EVANS, H. S. JACKSON, A. COOK, AND W. M. JOHNSTONE. *Can. Dairy Ice Cream J.*, 26, 9: 35-40. Sept., 1947.

A study is reported of the extent to which the water-soluble vitamins, riboflavin and thiamine, and the minerals calcium and phosphorus are retained in cheesemaking. Of the original calcium present in the milk, about 61% was retained in raw-milk Cheddar cheese. Of the original phosphorus, about 53% was accounted for in the cheese. These values did not vary with season. About 23% of the riboflavin originally present in the milk was retained in the cheese. The results with pasteurized milk ("holder" and HTST methods) indicate that the heat treatment did not noticeably affect the retention of calcium. Cheese made from pasteurized

milk tended to retain slightly more of the phosphorus than did cheese made from raw milk. Pasteurization had no significant effect upon the retention of riboflavin when compared with the raw control.

The calcium, phosphorus and riboflavin contents of a limited number of batches of cream, cottage, brick and blue cheese were reported. Cream cheese contained 84.4 mg.% calcium, 86 mg.% phosphorus, and 280 mg. per 100 g. of riboflavin. Cottage cheese contained 85 mg.% calcium, 146 mg.% phosphorus and 288 mg. per 100 g. of riboflavin. In brick cheese, of the original nutrients present in the milk, 57.7% of the calcium, 58.7% of the phosphorus and 27.4% of the riboflavin were retained in the cheese. In blue cheese, the corresponding values for retention were: calcium 46.2%, phosphorus 43.3% and riboflavin 30.1%.

Cheddar cheese showed little or no loss of thiamine by either the holder or the "High-Short" process. The process of cheese manufacture caused no actual destruction of thiamine.

H. Pyenson

297. Report on sampling, fat and moisture in cheese. WILLIAM HORWITZ AND LILA KNUDSEN, Food and Drug Administration, Minneapolis, Minn. J. Assoc. Offic. Agr. Chemists, 31, 2: 300-306. 1948.

A collaborative study was made of two methods for determining the moisture and fat contents of process American, rindless Cheddar and daisy Cheddar cheese. Moisture was determined by the official method and the force draft oven method, and the fat was determined by direct weighing of sample into a Mojonnier tube and by the official method. The average standard deviation of the official method for moisture determination was 0.23 and for the forced draft oven method 0.22. The average standard deviation for the Mojonnier tube method of determining fat was 0.44 and for the official method 0.48. Variation between samples of the same cheese at one laboratory was negligible, compared to the variation from one laboratory to another. The results indicated that the sample of shredded cheese sent to the collaborators was homogeneous and the differences obtained were not due to a difference between the samples sent to them.

F. J. Babel

298. Research problems in relation to Cheddar cheese quality. E. G. Hood. Can. Dairy Ice Cream J., 27, 4: 42. April, 1948.

The research projects under study are: (a) cause and control of such flavor defects as rancid, unclean and fruity; (b) mechanical openness; (c) control of extraneous matter in cheddar cheese; (d) clarification of milk in relation to quality; (e) starter problems; (f) bacteriophage in relation to cheesemaking; and (g) factors involved in the manufacture and storage of high quality cheese.

H. Pyenson

FOOD VALUE OF DAIRY PRODUCTS

299. Report on the phosphatase test in pasteurization of dairy products.

GEORGE P. SANDERS, Bureau of Dairy Industry, Washington, D. C.
J. Assoc. Offic. Agr. Chemists, 31, 2: 306-318. 1948.

Results indicated that the modified Kay-Graham procedure could not be adapted satisfactorily as an index of pasteurization in testing cheese. A description is given of the laboratory method of Sanders and Sager for testing various dairy products to determine the adequacy of pasteurization. The method includes modifications needed to produce uniformly quantitative results under fixed conditions in applying the test to various common varieties of cheese, fluid milk, cream, ice cream mix, sherbet mix, chocolate drink, butter, sweet buttermilk, cultured buttermilk, fermented milk drinks, goats' milk and cheese whey.

Phosphatase activity caused by microorganisms was not encountered in any fresh or reasonably fresh products. It was encountered in some samples of old butter, old cream, surfaces of soft and semi-soft ripened cheeses and in several specific cultures of microorganisms.

The substrate *p*-nitrophenyl phosphate decomposed relatively rapidly under the influence of heat when the controls and tests were heated after incubation. Even less precision was obtained with phenolphthalein phosphate than *p*-nitrophenyl phosphate.

The Sanders-Sager method was recommended by the Associate Referee as the official method for testing fluid milk and cream, cheddar type cheese and soft unripened cheeses for the index of adequacy of pasteurization. Also, he recommended that this method be made tentative for other types of cheese, ice cream mix, sherbet mix, chocolate drink, butter, sweet buttermilk, cultured buttermilk, fermented milk drinks, goats' milk, cheese whey and concentrated milk products. It was recommended that the present phosphatase test for pasteurization be dropped. F. J. Babel

300. Combination of formaldehyde with casein. A. P. SWAIN, ELSIE L.

KOKES, N. J. HIPP, J. L. WOOD, AND R. W. JACKSON, Eastern
Regional Res. Lab., U. S. Dept. of Agr., Philadelphia 18, Pa. Ind.
Eng. Chem., 40, 3: 465-469. March, 1948.

Graphs are presented to show the effects of concentration of formaldehyde, pH, time, and temperature on the amount of recoverable formaldehyde remaining in combination with casein after exhaustive washing of the reaction product with distilled water. The results are compared with related data of other investigators and are discussed in terms of possible reactions of various structural units in the protein. The analytical procedures employed for distillation and titration of recoverable formaldehyde

were extensively studied and improved. Experiments also are described that show appreciable conversion of formaldehyde to the nonrecoverable form in the presence of casein at 100° C. and above. B. H. Webb

301. Vitamin A and carotenoids in the blood serum of dairy cattle. Chemical methods for determination. D. B. PARRISH, G. H. WISE, AND J. S. HUGHES. Kansas Agr. Expt. Sta., Manhattan. Analyt. Chem., 20, 3: 230-233. March, 1948.

Four methods for the determination of vitamin A and carotenoids were compared. When cows received large amounts of vitamin A supplements, the results of the vitamin A determination of blood serum were too low if, without preliminary saponification, a method was employed that utilized carotene precipitation for the removal of interfering substances. Certain components of blood serum in addition to carotene interfered with the determination of vitamin A by the Carr-Price reaction. The interfering substances were susceptible to saponification and to milk oxidation.

B. H. Webb

302. Factors affecting the keeping quality of dried milk powder. R. A. CHAPMAN. Can. Dairy Ice Cream J., 27, 4: 45-46. April, 1948.

Good quality milk powders can be stored satisfactorily for periods up to 2-3 years if they are gas packed in an atmosphere containing 3% or less of oxygen. The solubility will not be impaired if the moisture content is kept below 3%. Ethyl gallate has been found very effective, either independently or in conjunction with a pre-heat treatment, in inhibiting oxidative deterioration.

H. Pyenson

303. Experimental enterococcal food poisoning in man. A. G. OSLER, L. BUCHBINDER, AND G. I. STEFFEN. Proc. Soc. Exptl. Biol. Med., 67: 456-459. 1948.

Symptoms of acute gastric or intestinal disturbance or both were produced in 6, or possible 7, of 26 human volunteers who consumed egg salad, custard, or milk in which single strains of *Streptococcus fecalis* had grown for 5 hrs. Attempts to produce similar symptoms in man with 20-hr. cultures grown in milk or in infusion broth, were unsuccessful. I. Peters

304. Mineral metabolism studies in dairy cattle. III. Manganese metabolism in the lactating bovine. J. THOMAS REID AND GEORGE M. WARD. N. J. Agr. Expt. Sta., Sussex. J. Nutrition, 35, 5: 591-596. May 10, 1948.

With manganese intakes ranging from 622.4 to 1325.6 mg. daily, cows

retained about 154 mg. daily during the first 5 months of lactation. The fecal elimination of manganese was proportional to intakes within the above range. Manganese in the form of manganese sulfate was utilized as well as the manganese of feed.

R. K. Waugh

305. The nutritional value of cheese to the consumer as compared with the price of milk. A. L. GIBSON. *Can. Dairy Ice Cream J.*, 27, 4: 62-66. April, 1948.

A method for calculating the nutritional value of cheese to the consumer on the basis of the retail price of milk is given in detail. The constituents of milk and cheese are converted to a common unit basis. The unit used is lactose. Using this unit system, tables are given comparing the value of 1 lb. of cheese to the consumer as compared with the retail price of milk. This method offers a sound system of cost accounting. H. Pyenson

ICE CREAM

306. Emulsifying and stabilizing agents for ice cream. LAWRENCE L. LITTLE, E. F. Drew Co., Boonton, N. J. *Milk Plant Monthly*, 37, 6: 42-48, 50. 1948.

The author describes fully the role of the basic stabilizers, such as gelatin, sodium alginate, vegetable gums and cellulose gum in ice cream as well as emulsifying agents. The emulsifying agent discussed is restricted to the fat-soluble type which is a derivative of a natural fat in which the fat has been modified so as to form a water soluble group in the molecule. The hydrophilic triglyceride added to the ice cream mix upon homogenization is oriented so that the water soluble groups are at the surface of the fat globules, thus forming a water soluble coating on the surface of the fat globules binding a film of water around the fat and holding it as water of hydration. In this way the hydrophilic triglyceride has stabilizing properties in that it (a) reduces the amount of water that will be converted into ice when the mix is frozen and hardened, (b) the hydrated fat globules function as hydrated colloid particles in deflecting the growth of large ice crystals into more numerous and smaller crystals and (c) the fat globules, hydrated as a result of the action of the hydrophilic triglyceride, counteract the dehydrating effects of freezing upon the stabilizer and milk proteins. Probably the most important function of an emulsifying agent in ice cream mix is its ability to increase the cohesion of the mix as a result of its property of holding a film of bound water around fat globules and the ability to retain this bound water through the freezing and hardening operations.

G. M. Trout

307. Emulsifiers—how they improve ice cream. H. L. CASLER, Germantown Manufacturing Co., Philadelphia, Pa. *Ice Cream Rev.*, 31, 10: 52, 54. May, 1948.

Emulsifiers supplied to the ice cream industry belong to that class of chemical compounds known as "esters." These are combinations of long chain fatty acids such as palmitic, stearic or oleic, and a higher alcohol such as glycerol or sorbitol. They have an affinity for both water and fat. The fatty acid end of the molecule is soluble in fat, whereas the alcohol is soluble in water. Furthermore, they are powerful surface active agents, *i.e.*, they move to any interface where fat and water come together and greatly reduce surface tension, which is the force that tends to cause fat to form the largest possible masses. The homogenizer easily can reduce the butterfat to sub-microscopic globules with the surface tension force removed. The result is a finer textured ice cream. Finally, the esters spread out over all interfaces until they entirely surround the tiny fat globules, thus forming a protective film which in conjunction with the stabilizer prevents clumping of the fat globules. Much the same action is believed to occur around the air cells of the finished ice cream, thus improving overrun and combating shrinkage. Since the dispersing and protective actions have no connection with low temperature, better and smoother melt-downs usually result.

Emulsifiers do not take the place of a stabilizer. When a combination emulsifier and stabilizer is purchased, the ratio between the two usually is correct. When emulsifiers are purchased by themselves, the usual amount of stabilizer should be used and the optimum amount of emulsifier should be determined by trial. Emulsifiers will give results only in a pasteurized and homogenized mix. Only at pasteurizing temperatures does the complete and intimate contact take place which is necessary for surface action; the resulting lowered surface tension merely is an aid to better homogenization. It is the homogenizer and emulsifier together which improves the emulsion.

Use of emulsifiers in the margarine industry dates back to 1920, and they are commonly used in the baking and confectionery industries. They are considered safe and have been proved to be non-toxic. In the purchase of an emulsifier for use in ice cream, it is important that a product be selected which is fully edible and which is completely free from objectionable flavors or odors.

W. J. Caulfield

308. Mix stabilizers and whipping agents in making ice cream. P. H. TRACY. *Can. Dairy Ice Cream J.*, 26, 10: 42-46. Oct., 1947.

The sources, manufacture and characteristics of the following mix stabilizers and whipping agent are discussed: (a) gelatin; (b) Irish moss;

(c) carob bean; (d) pectin; (e) Dariloid; (f) quince seed extract; (g) gums; (h) sodium carboxymethylcellulose and (i) emulsifying agents.

H. Pyenson

309. Ice cream shrinkage. H. A. BENDIXEN. *Can. Dairy Ice Cream J.*, 27, 4: 54-60. April, 1948.

The following precautions would be helpful in guarding against ice cream shrinkage: (a) avoid freezing the ice cream too stiff in the continuous freezer; (b) avoid extreme temperature changes or heat shocking; (c) use high-quality low-acid dairy products to prevent destabilization of the proteins; (d) avoid an excessively high sugar content and especially a high dextrose content; and (e) avoid the use of unparaffined cartons or cans, banging of the packages, and excessive air circulation directly over the ice cream in the storage room.

H. Pyenson

310. Quality in ice cream. E. L. WALKER. *Can. Dairy Ice Cream J.*, 26, 9: 31. Sept., 1947.

As high a quality of ice cream can be made with low fat content as with high fat content. There is usually a greater per capita consumption of ice cream where a high quality of ice cream is produced. Checking every product of ice cream for test, taste, color, flavor and texture before and after it is manufactured and before it leaves the plant should be a standard procedure.

H. Pyenson

311. Formula for the future. W. GRIFFITH. *Ice Cream Trade J.*, 44, 5: 42, 43, 92-95. May, 1948.

With ice cream sales going down in the face of increasing national income, the author makes the following 4 suggestions for turning ice cream sales upward: Make a lower butterfat, quality product; go after the packaged market; clean up the dealer's store; and get Johnny's and Mary's nickel.

W. H. Martin

312. High temperature-short time pasteurization of ice cream mix. C. M. MINTHORN, Chester Dairy Supply Co. *Ice Cream Trade J.*, 44, 5: 70, 70B, 99, 100. May, 1948.

Lack of suitable equipment has delayed the general use of high-temperature short-time pasteurization of ice cream mix. In plants where no condensing is done and only concentrated products are used, the heating of the mix must be done in two stages. The ingredients are placed in a mixing vat, pumped through a tubular heater, and heated to 125° F. before the final pasteurization treatment. In plants using raw products, and

where the condensing operation is a part of the mix-making system, the ingredients are mixed and pumped through a tubular heater before they pass to the vacuum pan for concentration to about 40% solids; this mixture is pumped into measuring tanks before final pasteurization. In the final operation the mix is picked up from the balance tanks with a centrifugal pump and pumped through a filter and through the first 16 tubes of another heater, where the temperature is raised from 120 to 160° F. From here the mix goes to 2 homogenizers, one of 1,250-gallon capacity and the other 400-gallon capacity per hour, where the mix is homogenized at 160° F. The discharges from these 2 homogenizers converge into a return line and go back to the heater, where the temperature is raised to 176° F. through the last 8 tubes of the heater. From this point, it goes to the holding tube, where it is held 22 seconds before going to the ice cream mix cooler. In this process the mix is heated to 160° F. before it goes to the homogenizers, which are metering pumps. The centrifugal pump is of slightly greater capacity than the homogenizers so that there is a continuous pressure in the suction side of the homogenizer so that no air is incorporated in the mix.

W. H. Martin

313. Latest developments in hardening ice cream. HARRY BITTERS. *Can. Dairy Ice Cream J.*, 26, 10: 66. Oct., 1947.

With the new method of filling direct in the carton, it is necessary to harden at a much faster pace to keep up with the capacity of the freezer. A freezing tunnel with a temperature of at least -40° F. with a blast of air circulated over the conveyor carrying the packages works satisfactorily. Four 30-in. high-speed fans can harden pint packages in 70 minutes at a capacity of 375 gallons per hr. The conveyor has a variable speed drive. The disadvantages are: (a) the oil in bearings and transmission is very likely to solidify; (b) leaks cause loss of refrigeration; (c) the formation of ice around the conveyor interferes with the operation. Defrosting is best accomplished by the use of hot gas.

H. Pyenson

314. Chocolate ice cream. R. A. SIMONET, Robert A. Johnston Co., Milwaukee, Wis. *Ice Cream Rev.*, 31, 10: 56, 58, 59. May, 1948.

The ideal combination for flavoring chocolate ice cream is a mixture of equal parts of chocolate liquor and cocoa. Such a mixture will have a fat content of 34% and a melting point of 92° F. The chocolate mixture plus 2% additional sugar should be incorporated into the mix at the pasteurizer. When homogenized at 1,500-2,000 lb. pressure, the possibility of specks in the finished ice cream is eliminated. Overnight aging will enhance a mellow chocolate flavor free of sharpness.

Straight chocolate liquor is not recommended for use in ice cream be-

cause it does not carry sufficient color and cannot be conveniently prepared as a paste for addition at the freezer. Cocoa does not carry enough of the high melting cocoa fat and the flavor does not remain long enough in the mouth. The proposed mixture with a melting point of 92° F. will, on the other hand, linger on the taste buds of the mouth long after the ice cream has been swallowed.

Caution is urged against the use of a chocolate product which is too acid or alkaline. If a too highly dutched product is used, the ice cream is apt to have a dull muddy appearance and may develop greenish spots if stored in poorly tinned metal cans without paper liners. Procedures are outlined for the production of variegated chocolate ice cream with both the batch and continuous freezers. It is strongly recommended that a weekly tasting panel be set up to examine all chocolate flavored ice creams being offered for sale and to determine what adjustments, if any, should be made to satisfy local market demands. W. J. Caulfield

315. Frozen berry purees and their application in the dairy industry.

E. H. WIEGAND. *Can. Dairy Ice Cream J.*, 26, 9:41-43. Sept., 1947.

Soft ripe fruit is used in the manufacture of purees to obtain an excellent product of high flavor. Hard fruit lacks flavor and may produce a bitter puree. The two types of purees suitable for ices and ice cream are the simple frozen puree and the pectinized puree. The fruit should be pulped at a low temperature (35° F.) to prevent oxidation. Thorough deaeration is essential to produce purees of better keeping quality. The fruit is cleaned, peeled, if necessary, crushed and frozen in barrels or enamel-lined cans. Simple purees are suitable for sherbets, ices, Velva fruit, and soda fountain flavors and pectinized purees for ribbon or ripple ice cream. Formulas are given for the preparation of pectinized purees from various berries, pears, nectarine, apricot, Velva fruit, high acid-low pectin content fruits and low acid-high pectin content fruits. H. Pyenson

316. Evaluating the flavor of ice cream. D. V. JOSEPHSON. *Can. Dairy Ice Cream J.*, 27, 5: 50-56. May, 1948.

Apart from individual preferences, the composition of ice cream is sometimes dictated by the economic and racial status of a community. The total flavor response is the result of the 3 separate functions of the taste mechanism, taste, touch and smell. All people do not have the same degree of development or refinement in their taste functions. The over-all flavor of ice cream is a delicate blend of sweet and salty taste responses coupled with the olfactory response characteristic of the particular flavor in question. The tactual taste receptors on the tongue contribute to the over-all flavor by recording the texture and "feel" sensation in the mouth.

Some of the factors that influence the efficiency of taste work are sensitivities and patterns of taste response of the judges; scoring ranges are too wide and do not permit an accurate or tangible evaluation of the product; a key or dominant figure whose scores involuntarily become the standard for the group; choosing a time for judging samples; too many samples are included in each judging session; and taste panels evaluate ice cream in terms of their own personal opinion which may not necessarily represent the average consumer. Some suggestions for improving tasting techniques are as follows: (a) calibrate each member of the taste panel and determine their relative sensitivities or thresholds to basic, abnormal and deteriorative flavor qualities; (b) a maximum range of 5 to 6 points should be employed; (c) every judge should have complete freedom in expressing his judgments; (d) establish a standard and uniform terminology for describing the flavors and textures of ice cream; (e) have ample time for judging; (f) judge in room where there are no disturbances or noise; (g) evaluate products in terms of consumer's preference; (h) delete identification marks on packages; and (i) a member of the taste panel should not set up the samples.

H. Pyenson

317. A consumer taste-test panel. ANONYMOUS. *Ice Cream Trade J.*, 44, 5: 34-35, 95, 96. May, 1948.

A consumer clinic which regularly tests ice cream has been set up by a dairy laboratory at Scranton, Pa. The tasters who form the panel are chosen from among professional and business people, housewives and nutritionists; participation is by invitation only. "Judgment sheets" calling for scores on appearance, body, texture, and flavor, along with other comments or criticisms, are used by the testers. These independent taste panels provide a guide for the manufacturer to use in the determination of the quality of his ice cream. Opinions of these impartial judges can help to wipe out many fallacies that exist in the consumer's mind. A copy of the completed clinical report is furnished to the ice cream manufacturer subscribing to the clinic, and he also receives a laboratory and bacterial analysis showing the percentage of fat, total solids, acidity, homogenization index and standard plate and coliform counts on the ice cream.

W. H. Martin

318. Stick novelties operation in medium-sized plant. T. G. MUNROE. *Can. Dairy Ice Cream J.*, 27, 5: 66-70. May, 1948.

All products, equipment and methods were standardized after the early patent rights were acquired, and this material was controlled by a single management. In order to produce stick novelties successfully, it is necessary to have proper equipment (brine tank), filling molds, sticking by ma-

chine, defrosting of molds quickly, chill or drying tunnel and dipping, bagging, and packing equipment. Some of the most important points in stick novelties operation are keeping the operations up to date with the latest money-saving equipment of proper capacities, streamline the operations, prevent waste of material, cartons, bags, sticks and coating, and use effective advertising in the dealers' stores.

H. Pyenson

319. Trends in the ice cream business. J. L. DOLPHIN. *Can. Dairy Ice Cream J.*, 27, 4: 32-36. April, 1948.

Trends in the manufacture and sale of ice cream and suggestions for developing marketing of ice cream are discussed. The subjects reviewed are batch freezers, continuous freezers, shrinkage, brick ice cream, overrun, deep freeze boxes, packed ice cream, advantages of pint packages, and advantages of round package.

H. Pyenson

320. New Jersey ice cream men campaign against sale of ice cream by weight. ANONYMOUS. *Ice Cream Rev.*, 31, 10: 74, 76, 78, 80, 82. May, 1948.

Some of the reasons advanced against the sale of ice cream by weight are: (a) It is impossible to produce ice cream with a uniform weight per gallon. The weight per unit value is not only influenced by the per cent overrun, but by the composition of mix, the added flavors, etc. Since no tolerance is allowed in the measurement of products sold by weight under the New Jersey law, each package would have to be weighed individually and have its exact weight stamped on the container. This would necessitate extra labor and inventory records would be more complicated. (b) The weight system would reduce the efficiency of the individual truck driver. More time would be involved in checking out his load at the plant and in billing dealers for goods received. (c) Extra costs involved in producing ice cream by weight would of necessity have to be passed on to the consumer. (d) Sale of ice cream by weight by some 15,000 retailers in New Jersey would necessitate the purchase of special scales and would slow up service. (e) Sale of ice cream by weight does not mean the consumer will receive more value for his money. On the contrary, the practice probably would lead to the production of an ice cream with a minimum fat content, with the weight increased by the addition of extra sugar solids. (f) Retailers and the public are protected against receiving ice cream with excessive amounts of overrun by the present New Jersey law, which requires that ice cream be manufactured with a minimum weight of 4.5 lb. per gallon. (g) Public enforcement of fair measure is much more feasible on a volume basis than on a weight basis. The consumer can see when a container is not properly filled, but few will have scales to check the

weight of the product. (j) Sale of ice cream by weight would lead to untidiness and carelessness in weighing out the various packages or servings at soda fountains. (k) The sale of ice cream by weight has not proved successful where it has been tried. The experiment of selling ice cream by weight lasted for less than a year in Los Angeles, and was discontinued at the request of the druggists association which had sponsored the move. (l) The majority of dealers in New Jersey oppose the sale of ice cream by weight. (m) There is no great public demand for the sale of ice cream by weight. Despite the fact that the New Jersey law now permits the sale of ice cream by weight, only a handful of dealers have availed themselves of this privilege.

The ice cream manufacturers of New Jersey make it clear that they are not opposed to a law against the sale of ice cream by weight if a just, workable law could be devised. They do oppose a law which would work a hardship on the public and discriminate against the manufacturers and dealers.

W. J. Caulfield

MILK

321. What constitutes quality in milk. G. M. TROUT. *Can. Dairy Ice Cream J.*, 26, 9: 32-34. Sept., 1947.

Different quality factors are emphasized by various groups. The various groups to consider are the consumer, the producer, the processor and distributor, the bureau of standards inspector and the milk sanitarian or health officer. The following factors enter into a definition of quality milk: (a) cream line; (b) appearance; (c) safety; (d) cleanliness; (e) good flavor; (f) keeping quality; (g) nutritive quality; (h) low bacteria count; (i) esthetic background of production; (j) composition; and (k) miscellaneous, such as freedom from adulterants, no watering, etc.

H. Pyenson

322. The time factor in high-temperature short-time pasteurizing. H. B. ROBINSON AND C. M. MOSS, U. S. Public Health Service, Dist. 1, New York City. *J. Milk and Food Technol.*, 11, 1: 44-51. Jan.-Feb., 1948.

The U. S. Public Health Service Milk Ordinance and many state and local regulations specify 160° F. for 15 seconds for high-temperature short-time pasteurization. The specifications include a safety zone of at least 9 seconds at 160° F. and conversely of at least 5° F. if the time is 15 seconds. There appears to be no need for increasing either the time or temperature beyond those margins established by the fluctuations of the pasteurizer controls.

Large errors are inevitable where water runs are used in timing high-temperature short-time units. A variation of 26.7% or more was noted when water and milk were delivered to the same pump. Therefore milk run tests should be used in timing. Both the volume delivered and temperature fluctuation technics appear to have application in extending water run tests to milk by empirical correction.

H. H. Weiser

323. Homogenized milk problems. P. H. TRACY, Univ. of Ill., Urbana. Milk Plant Monthly, 37, 6: 58-68. 1948.

Problems encountered with homogenization of milk include sufficient breakdown of the fat globules to prevent fat rising, curd tension reduction, prevention of sediment formation, maintenance of low bacterial content of the homogenized milk, testing for fat by the Babcock method, curdling during cookery and flavor defects—particularly rancid and sunlight flavors. These defects can be overcome by carrying out the homogenization process effectively through using sufficient pressure, maintaining proper temperatures and having valves in good condition. Many data are presented showing the effects of numerous processes on sedimentation in homogenized milk. If the cell content of non-homogenized milk is less than 200,000 per ml., likely the homogenized milk will not show enough sediment to be of any consequence. A low bacterial count can be obtained by washing and sterilizing the homogenizer after each day's use according to prescribed procedures. Especial attention must be paid to keeping homogenized milk out of sunlight if the sunshine flavor is to be prevented.

G. M. Trout

324. Problems on quality milk production. C. D. MACKENZIE. Can. Dairy Ice Cream J., 27, 4: 39-40. April, 1948.

The problems relating to high quality milk may be divided into those dealing with cleanliness of milk, such as disease and management, and those dealing with the nutritive value of the product as affected by breeding and feeding. The diseases important to eliminate for high quality milk production are mastitis, Bang's disease and tuberculosis. The relation of management, sanitation, breeding and feeding is discussed in relation to quality milk production.

H. Pyenson

325. Should we have a simple standard of milk quality. G. M. TROUT. Can. Dairy Ice Cream J., 26, 10: 60-62. Oct., 1947.

Recent advances in production, distribution and public health point more and more toward the realization of a simple standard of quality for milk.

H. Pyenson

326. Prospective milk production and consumption in Canada. B. L. CAMPBELL. *Can. Dairy Ice Cream J.*, 27, 3: 68-72. March, 1948.

A study was made of prospective trends based on dairy, agricultural and business statistics and an interpretation of these statistics are given.

H. Pyenson

327. Sanitation and its control. G. E. STANLEY. *Can. Dairy Ice Cream J.* 26, 10: 38-40. Oct., 1947.

There has been a tremendous expansion in the consumption of milk during recent years, but very little has been done to improve the sanitary quality of the raw product. The article discusses bacterial counts, mastitis, bacteriological surveys and laboratory methods of control.

H. Pyenson

328. Farm inspection for better milk supply. A. E. BERRY. *Can. Dairy Ice Cream J.*, 27, 2: 64-70. Feb., 1948.

Looking back over a period of 30 years in Canada, the article discusses: (a) control of disease; (b) accomplishments of pasteurization; (c) legislation and administration; (d) new health units; (e) qualification of personnel; (f) farm inspection by veterinarians; (g) bacterial standards; (h) standard for pasteurized milks; (i) factors in farm inspection; (j) cleaning of utensils and (k) cooperation of distributors.

H. Pyenson

SANITATION AND CLEANSING

329. Bottle washing problems. D. H. JACOBSON. *Can. Dairy Ice Cream J.*, 26, 9: 44-50. Sept., 1947.

Efficient sterilization of bottles is obtained by the use of proper time, temperature and caustic strength. The problems in soaker bottle washing are: (a) efficient sterilization; (b) complete cleaning and bright appearance; (c) prevention of etching of glass or color labelling; (d) prevention of scale on the washer; and (e) smooth mechanical operation or lubrication. Methods of obtaining satisfactory results are discussed.

H. Pyenson

330. Can washing in creameries and milk manufacturing plants. P. J. BOGAERTS. *Can. Dairy Ice Cream J.*, 27, 3: 74-78. March, 1948.

Milk and cream cans must be sent out from the creamery or milk plant to the farm in an absolutely sterile condition whether the cleaning operations are conducted by hand or by machine can washing. The methods of cleaning mechanical washers are given in detail.

H. Pyenson

331. Cleaning and sterilizing of dairy equipment. J. S. GEORGE. Can. Dairy Ice Cream J., 26, 9: 52-53. Sept., 1947.

The old standard cleaning materials are discussed giving new applications for their use. These materials discussed are the carbonates or soda cleaners, silicates, phosphates, complex phosphates, caustic soda, washing compounds and acid cleaners. The new materials developed are the synthetic detergents and the quaternary ammonium compounds.

H. Pyenson

332. A rapid field test for quaternary ammonium salts used in germicides. R. F. BROOKS AND G. F. HUCKER, New York State Experiment Station, Geneva, N. Y. J. Milk and Food Technol., 11, 3: 136-138. May-June, 1948.

A rapid routine test for checking quaternary ammonium solutions has been proposed. It is not intended to be an accurate quantitative method. The method is based on the principle that an excess of a mixture of quaternary salts and ethylene dichloride in the presence of certain anionic indicators in acid solution produces a yellow-green color in the ethylene dichloride layer of the mixture. This gives a sharp contrast with the blue-violet color of the aqueous layer, making the end point more easily visible. Approximately 1 ml. of ethylene dichloride is added to 1 ml. of the quaternary ammonium solution in a small bore test tube. The tube is inverted to facilitate mixing. Bromphenol blue indicator (buffered at pH 4.5 to 4.8) is added (one drop to approx. 0.05 ml.) The contents of the tube are mixed by inverting after each addition of indicator and allowing the layers to separate before adding more indicator. The number of drops of indicator required to give a permanent yellow-green color in the ethylene dichloride (lower) layer as compared to a blue-violet color in the aqueous (upper) layer, indicates the concentration of quaternary ammonium solution.

H. H. Weiser

333. Quaternary ammonium and hypochlorite solutions for sanitizing dairy utensils and equipment. C. K. JOHNS. Can. Dairy Ice Cream J., 27, 3: 27-29. March, 1948.

The quaternary ammonium germicides have many desirable properties which make them suitable for use in the sanitizing of dairy utensils and equipment. In general, they were slightly more effective than the hypochlorites against gram-positive organisms; against gram-negative organisms, the reverse held true. Cheese starter organisms were killed more readily by hypochlorites. The type of water used to prepare dilutions of the two types of germicide appeared to have some influence. Tap water solutions of "QA" (one of the quaternaries) were more sensitive to added skim

milk than were distilled water solutions, while hypochlorites showed little difference. Hypochlorites were much less sensitive to added skim milk than is usually believed. While the quaternaries showed some response to favorable adjustments in pH and temperature of solution, the response was slight compared with that shown by hypochlorite. As preservatives in milk, quaternaries are far less effective than formaldehyde. H. Pyenson

334. Water treatment and the use of chemicals in the creamery. L. R. BRYANT. *Can. Dairy Ice Cream J.*, 27, 4: 27-30. April, 1948.

Lack of use, or improper use of water conditioners, water softeners, cleaning compounds and sterilizing chemicals can produce large losses through breakdown and replacement of equipment, lowered efficiency, inadequate cleaning and sterilizing of both equipment and containers and consequent lowering of product quality. The 3 main problems connected with the water supply in a creamery are: (a) scale on the surface of any heat exchange type of equipment; (b) corrosive action of some water; (c) source of water used for washing butter. The following methods of treating water are discussed: (a) silicate treatment; (b) chromate treatment; (c) complex phosphate threshold treatment; (d) water softening; (e) alkali cleaners; and (f) acid cleaners. H. Pyenson

335. Testing equipment as a practical aid to efficient sanitation. LEE H. MINOR. *Can. Dairy Ice Cream J.*, 26, 10: 48-50. Oct., 1947.

The service testing equipment that can be used readily to aid efficient sanitation includes: (a) a germicidal test kit for determining the amount of available chlorine in parts per million in germicidal rinse solutions; (b) a film tester for determining the source of film on equipment; (c) a control meter which automatically registers on a dial the concentration of a cleaning solution by percentage; (d) an alkometer for registering on a dial the percentage of caustic concentrations in the washing solution; (e) a titration test kit for making chemical tests and titrations; (f) germicidal test papers for quickly checking p.p.m. of available chlorine; and (g) alkacid test paper for checking a solution to determine whether acid or alkaline. H. Pyenson

MISCELLANEOUS

336. The effect of inanition on mammary-gland development and lactation. J. F. SYKES, T. R. WRENN, AND S. R. HALL, Bureau of Dairy Industry, USDA, Beltsville, Md. *J. Nutrition*, 35, 4: 467-476. April 10, 1948.

One group of rats was fed from weaning through pregnancy with feed

intake limited to 70% of that received by controls. At parturition, one-half of the rats in each group were sacrificed. Mammary gland weight at parturition averaged 3.34 g. for the control group and 1.87 g. for the restricted diet groups. Following parturition, all remaining rats were full fed, and allowed to suckle litters for 21 days, and then sacrificed. At the end of 21 days, litters of the control group had gained an average of 163.8 g., the litters of rats restricted during pregnancy 198.3 g. At the end of the suckling period average mammary gland weight for the control group was 283.5 g. and for the group restricted during pregnancy 220.4 g.

R. K. Waugh

337. Value of research to dairy industry. W. H. Cook. *Can. Dairy Ice Cream J.*, 26, 9: 27-30. Sept., 1947.

Research is any effort designed to yield something new, whether new knowledge, new processes or new products. Canadian industry might increase its expenditures for research to bring them more in line with the research expenditures made by industries in other countries. The article deals with (a) the cost of research, including some suggestions as to how the limited funds might be used to the best advantage; (b) the specific projects of the research program; and (c) the returns to be expected from these research expenditures.

H. Pyenson

338. Apparatus for homogenizing mixed liquid ingredients. J. B. McFADDEN. (Assigned to United Dairy Equipment Co.) U. S. Patent 2,441,711, May 18, 1948 (3 claims). *Official Gaz. U. S. Pat. Office*, 610, 3: 669. 1948.

Two discs, rotating within a casing and in opposite directions, have radially spaced concentric projecting blades, so disposed that intimate mixing of liquid ingredients is effected. Emulsification, comminution and mixing are accomplished by impacting the product against the sharp cutting edge of each blade, the product moving from one blade to another by centrifugal force.

R. Whitaker

339. Survey indicates continued trend toward converting boilers to oil or gas. ANONYMOUS. *Ice Cream Rev.*, 31, 10: 50. May, 1948.

A survey made in the South Atlantic, South Central, Mountain, and Pacific States revealed that 75.6% of the 243 ice cream firms replying to the questionnaire are already using gas or oil-fired boilers. Of the 7.5% of firms reporting they were contemplating a change in boiler fuels, 45.5% indicated they were planning to shift from coal to oil, 13.6% from coal to gas, and 9% indicated they planned to shift from oil to coal (because of greater economy and because of the uncertainty of oil deliveries). The ice cream industry has converted from coal to oil or gas to a greater extent than

have other branches of the dairy industry. Only 56.6% of the milk dealers and 34.6% of the creameries surveyed were using oil or gas.

Of 276 ice cream manufacturers replying to a questionnaire on the number of boilers, boiler capacity, and loads in their respective plants, over half of the plants had boiler capacities of less than 40 h.p., with the number of boilers per plant averaging 1.9. The boiler loads of over 75% of the plants varied from hour to hour and from day to day, whereas 24.6% of the plants indicated they operated with steady boiler loads. In over 60% of the plants, 90% or more of the steam generated was used for processing. Steam used for heating the building amounted to only 10% of the total generated in about half the plants and was less than 30% in over 90% of the plants.

The reasons most frequently given for conversion from coal to oil or gas were: cleaner, saves labor, convenient, economical and efficient. Before any contemplated changes from coal to oil are made, ice cream manufacturers are cautioned that they should first obtain the assurance of some responsible and reputable company that it will take care of their oil requirements on an equal basis in the amount estimated they will require.

W. J. Caulfield

340. New developments in the field of refrigeration. W. H. MARTIN. *Can. Dairy Ice Cream J.*, 26, 9: 60-66. Sept., 1947.

It is desirable to operate the refrigerating equipment as efficiently and economically as possible to maintain a high overall plant efficiency. Important points to remember are: (a) operate with as high an evaporating temperature as possible; (b) the coldest water available should be used for condensing purposes at a rate of about 3 U. S. gallons per ton-minute; (c) remove air or foreign gas from the system; (d) operate with as low discharge pressure and as high suction pressure as possible; (e) save refrigeration by regenerative processes and by using water. Other details discussed are evaporative condensers, moisture in the system and refrigeration controls.

H. Pyenson

341. Family consumption and dairy products. ANNA M. SPEERS. *Can. Dairy Ice Cream J.*, 27, 5: 42-48. May, 1948.

The consumption of dairy products depends upon national income, educational efforts, food expenditure, number of adults and number of children in the household. Milk utilization is a factor in the amount of milk used. The consumer wants safe milk, milk labelled as to grade, and square containers, preferably glass. In a sample group study of 70 families that average \$1,820 per year, as prices rose, they began to stop buying clothing, household furnishings and utensils, and decreased expenditures on recreational gifts. The largest portion of the budgets of these families is today being spent on food, shelter, fuel and light.

H. Pyenson

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

BOOK REVIEWS

342. *Advances in enzymology and related subjects of biochemistry*, vol. VIII. Edited by F. F. NORD. 538 pp. \$8.00. Interscience Publishers, Inc., New York, N. Y. 1948.

The 8 chapters in this volume are: Functioning of the cytoplasm; Quantitative studies on complement; Dehydropeptidases; Antifatty-liver factor of the pancreas—present status; Alkaloid biogenesis; Certain aspects of the microbiological degradation of cellulose; Synthesis of lipides; The biochemistry of fatty acid catabolism; Lipoxidase and the autoxidation of unsaturated fatty acids; and Enzymes of snake venom and their biological significance. Of the 14 authors, 5 are resident in foreign countries, thus contributing to the international flavor of the presentation. The presentation for each subject appears to be adequate and critical. The material is well-organized and well-indexed. This volume contains a cumulative index for all 8 volumes which have appeared to date. This is a valuable contribution in the struggle to summarize the increasing mass of scientific literature in forms which permit scientists to keep informed in areas outside of their limited specialties.

F. E. NELSON

343. *Manual of veterinary bacteriology*. RAYMOND A. KELSER AND HARRY W. SCHOENING. 5th edn. 767 pp. \$6.50. The Williams and Wilkins Co., Baltimore, Md. 1948.

The current edition of this book is a mixture of the up-to-date and the archaic in its field, the former predominating in some sections and the latter in others. Much material on specialized technics of value to bacteriologists handling pathogenic microorganisms, some of it not available from other common sources, is presented. The sections on general bacteriology and infection and immunity are the most in need of modernization. To mention a few specific examples, the particulate nature of bacteriophages and viruses is discussed in terms of diffusion experiments of 12-15 years ago, with no mention made of the numerous electron microscope micrographs and studies of the past 7 years. The different forms of penicillin receive no consideration. Except for two references to textbooks published in the middle thirties only one reference to work published after 1903 is given in the unexpectedly brief chapter on infection and immunity. Although the sixth edition of *Bergey's Manual* was published several months earlier

by the same publisher and the revised classification to be used had been publicized in some detail long before that, the classification employed, and thus the organization of the largest part of the book, is based upon the fifth edition of *Bergey*. In the chapter on milk and water bacteriology, bases for interpretation of results are lacking. Standards for coliform bacteria in either milk or water are not presented. Five lines, including one reference to an article published in 1935, are devoted to the phosphatase test, which might very well have occupied at least the half page devoted to the relatively unimportant color changes which bacteria may cause in milk. The illustrations are neither qualitatively nor quantitatively what one expects in a book published in 1948. The discussions on the microbiology of diseases which affect cattle are the portions which probably would be most useful to those in the dairy industry.

F. E. NELSON

344. *Bacteriology. A textbook of microorganisms.* F. W. TANNER AND F. W. TANNER, JR. 4th edn. 625 pp. \$4.50. John Wiley & Sons, Inc., New York, N. Y. 1948.

An appreciable amount of new material has been added in various places in the revision of this text, which treats bacteriology as such instead of as a branch of the medical profession. A tendency for more explanation and somewhat more concise organization than in earlier editions is apparent. The book is a good source of general information on microbiology.

Despite the generally favorable impression, several shortcomings which one hardly expects in a revision dated 1948 were encountered. The illustrations, on the whole, leave quite a bit to be desired, as many of them are either completely out of date or much inferior to what could be obtained without undue effort. By October 1947, when this book apparently went to press, the outline of the classification system used in the sixth edition of *Bergey's Manual of Determinative Bacteriology* had been publicized quite widely, so there seems little justification for retaining the outline from the fifth edition. A portion of the discussion of water bacteriology is based upon the 1933 edition of *Standard Methods for Water Analysis*.

F. E. NELSON

BACTERIOLOGY

345. *Inhibition of lactic acid bacteria by analogs of pantothenic acid.* WILLIAM DRELL AND M. S. DUNN. Univ. of California, Los Angeles. J. Am. Chem. Soc., 70, 6: 2057. 1948.

The growth of 23 strains of lactic acid bacteria, which require pantothenic acid, is inhibited by ω -methylpantothenic acid. The taurine analog showed less inhibitory action than the β -alanine derivative, but more than the derivative containing L-leucine. With four species of lactobacilli it was

shown that the inhibitory action of ω -methylpantothenic acid is reversed competitively by pantothenic acid over a wide range of concentrations.

H. J. PEPPLER

CHEESE

346. The manufacture of good quality cheese from pasteurized milk.
H. L. WILSON, Kraft Foods Co., Chicago, Ill. Southern Dairy Products J., 44, 1: 28-31. July, 1948.

It will not be too long before practically all Cheddar cheese produced in this country will be made from pasteurized milk. A cheesemaker has complete control of the making process when he has a good quality milk that is properly pasteurized.

The intake room must be properly supervised, every can of milk must be inspected and only milk that can be made into good quality cheese accepted. The milk should be heated just high enough and held just long enough to show a phosphatase-negative test. A standardized procedure such as is suggested in detail should be followed.

To make further progress, the cheese industry should have a program including: (a) properly constructed buildings, with up-to-date sanitary equipment kept in such condition that the milk and curd will not become contaminated; (b) a good quality of clean milk whether it is pasteurized or not; (c) a good active uniform starter; (d) approved uniform methods of manufacture; (e) curing conditions that will permit the continued activity of essential bacteria which develop the characteristic flavor of Cheddar cheese.

F. W. BENNETT

CHEMISTRY

347. Recent developments in the vitamin A field. IAN HEILBRON. J. Chem. Soc., 1948: 386-393. March, 1948.

This material is the text of a lecture reviewing the chemistry of vitamin A, particularly from the standpoint of syntheses of the various compounds important in elucidation of structure and function.

F. E. NELSON

348. Structural relationships in the natural unsaturated higher fatty acids. T. P. HILDITCH. J. Chem. Soc., 1948: 243-252. Feb., 1948.

This is the text of a lecture reviewing the structure and occurrence of these acids, particularly with respect to aquatic life and seeds.

F. E. NELSON

CONCENTRATED AND DRY MILK; BY-PRODUCTS

349. Dry milkfat as a form of storage fat. R. J. REMALEY, Kraft Foods Co., Chicago, Ill. Southern Dairy Products J., 43, 5: 39, 46. May, 1948.

Various methods of preparation of dry milkfat have appeared in the literature. A practical method of production is described in the U. S. Patent 2,406,819 issued to Arthur W. Farrall and assigned to the Creamery Package Manufacturing Company.

Dry milkfat is produced from fresh cream. The principle involved is removal of water and serum solids from the cream by centrifugation. The cream is heated to pasteurizing temperature, preferably 170 to 190°, and separated into 80% fat. The plastic cream then is run through an homogenizer and into a continuous-type settling tank. The resulting product is approximately 98% fat. It is decanted and passed through an additional centrifuge to produce a dehydrated milkfat.

Standards of the U. S. Quartermaster Corps are: milkfat, not less than 99.8%; moisture, not more than 0.1%; copper, not more than 0.25 p.p.m.; peroxide value, zero; free fatty acids, not more than 0.5. Such dry milkfat can be stored at 40° F. for 6 months with little analytical or organoleptic change. At 0° it will remain in excellent condition for an indefinite period of time. Dry milkfat may be used any place that butter can be used.

F. W. BENNETT

FEEDS AND FEEDING

350. Preliminary observations on using a synthetic milk for raising pigs from birth. L. K. BUSTAD, W. E. HAMS, AND T. J. CUNHA, State College of Washington, Pullman. Arch. Biochem., 17: 249. 1948.

An attempt was made to raise pigs removed from the mother at birth and placed on a synthetic milk containing all known vitamins, crude casein, a liver preparation, brewer's yeast, lactose, and colostrum substitutes. Pigs fed no colostrum substitutes (blood serum and plasma) died shortly after birth. Severe diarrhea occurred in all pigs; it could not be controlled with penicillin, sulfathalidine, sulfamethazine and Kaopectate. The obviously inadequate diet failed to keep pigs alive more than 22 days.

H. J. PEPPLER

FOOD VALUE OF DAIRY PRODUCTS

351. Sugar combined with milk in ice cream no tooth decay threat. ANONYMOUS. Ice Cream Rev. 31, 11: 160. June, 1948.

Sugar, when combined with milk or dry skimmilk and butter, is not a

contributing cause to dental caries, according to results of a study recently conducted at the University of Wisconsin.

In experiments with cotton rats, milk was found to protect the teeth of rats against dental caries and afforded a certain degree of protection, even when the rats were fed a solid dry ration, which characteristically produced a great many carious lesions. When dry whole milk, dry skim-milk plus butter or casein-sucrose experimental diet was reconstituted with water and fed, a great reduction in dental caries was noted. It was concluded that a large part of the protective effect of milk is due to its fluidity. Other observations indicated particle size to be important in preventing tooth decay, since rations made up of very coarse sugar caused less dental decay than did rations of finely powdered sugar. W. J. CAULFIELD

ICE CREAM

352. Concentrated and dry ice cream mixes. R. J. REMALEY, Kraft Foods Co., Chicago, Ill. Southern Dairy Products J., 43, 2: 110-11. Feb., 1948.

During 1945 approximately 100,000,000 lb. of dry and concentrated ice cream mixes were shipped to the armed forces. Tests indicated that the maximum length of time they would stand up at 100° F. was 4 months. The use of ice cream mix by the housewife and small ice cream manufacturers still is increasing.

Ice cream mixes have an excellent future, provided (a) high quality dry or concentrated mix is maintained by the manufacturer; (b) the manufacturer and user rotates his product and does not extent periods of storage excessively; (c) unnecessary regulations are removed from present legislation and new workable regulations are set up; (d) a realistic attitude toward the economy of exportation is maintained. F. W. BENNETT

353. New flavors for ice cream. F. W. BENNETT, University of Georgia, Athens. Southern Dairy Products J., 41, 2: 55. Feb., 1947.

Two new flavors for ice cream are described, muscadine and peanut butter. The muscadines are prepared by washing and removing foreign material which may be present. The grapes then are pressed to remove most of the pulp and juice, leaving hulls and seeds. The hulls are heated to tenderize and passed through a colander to remove the seeds, after which they are combined with the pulp and juice. One lb. of sugar is added for each 4 lb. of fruit. Two or 3 quarts of the sweetened fruit is sufficient for 10 gallons of ice cream. The sweetened fruit also may be stored indefinitely at 0° F. or lower.

Some brands of peanut butter are superior to others for use in ice cream.

Two lbs. of the product is the proper amount to flavor 10 gallons of ice cream. If the butter is mixed with 1 lb. of dry sugar before adding to the freezer, its even distribution will be facilitated. Both flavors have been well received by local consumers.

F. W. BENNETT

354. Factory-produced carry-out sundaes. E. THOM, Assoc. Ed., *Ice Cream Review*. *Ice Cream Rev.*, 31, 11: 44, 45, 96. June, 1948.

The carry-out sundae, produced on a production line basis, is one means by which ice cream gallonage may be increased. At the Herron Ice Cream Company in Cleveland, a foursome package currently is being produced with considerable success. The package, approximately equivalent to 1 pint, consists of 4 ice cream rolls, approximately 2 inches in diameter, with a center core of fruit and pectin and a sprinkling of ground nuts, macaroons or almond bisque on the top.

A stainless steel extruder pipe is attached to the continuous freezer for producing the ice cream portion of the roll and the fruit is pumped to form the core through an inner stainless steel tube of any shape desired. Club, heart, diamond and spade combinations have proven to be the most popular core designs. The ice cream roll with its center core as it emerges from the 2 tubes, one inside the other, is fed onto stainless steel pans 3 ft. long. The pans move on a conveyor directly to the hardening room. Just before entering the hardening room, the pans pass beneath a device which sprinkles the top of the ice cream with ground macaroons or nuts, etc. After hardening, the ice cream roll is cut into any desired length. The individual portions then are placed into individual cellophane containers and packaged in units of 4 in cardboard boxes.

The package retailed for 35 cents in Cleveland at the time when ice cream was being sold for 30 cents a pint. This foursome package has proven extremely popular for sale directly into homes, and has enjoyed an excellent market among hotels, churches, lodges and other organizations holding large dinners. It has proven particularly helpful in keeping up sales during periods of low production.

The Esmond Dairy Company at Sandusky, Ohio, uses the same equipment to produce the sidewalk sundae. This item consists of a roll of ice cream with a fruit core, but without nuts or macaroons being added. The ice cream roll of suitable length is wrapped with paper, which is easily peeled off, and is then inserted in a cone. This item was introduced during the depression period to combat double- and triple-dipped cones.

W. J. CAULFIELD

355. The retail store of the future. D. GHORMLEY. *Ice Cream Trade J.*, 44, 6: 56. June, 1948.

The ice cream store of the future will be located in a suburban business

district within a few blocks of a middle-class residential district and on a main boulevard. It will be found in a specially constructed building, 35 x 60 feet, which will have a large plate glass window, a 12-foot ceiling in the store area, a mezzanine over the storage area and wash rooms for customers. The fixtures will cost between \$7,000 and \$9,000 and will be made of colored plastics and stainless steel. All food preparations will be done in the kitchen and picked up by waitresses through pass-out windows. Dish washing will be done in a separate room. The store will seat 37 to 43 and will have 4 booths.

Standardization of food preparations, salesmanship and the daily operating technics will be utilized. The company president will be an experienced retailer and constantly on the alert for new ideas. Many side-line products will be handled by the store of the future. The store will be air conditioned, and have an "electric eye" door and a concealed nickelodian. There will be no home delivery or credit offered. W. H. MARTIN

356. Borden's guides its dealers on retail selling prices. ANONYMOUS. Ice Cream Trade J., 44, 6: 46, 82-84. June, 1948.

The Borden Company letter announcing price increases on ice cream was accompanied by a brochure entitled, "How to Price Ice Cream." Prices recommended for hand-dipped half-pints, pints and quarts were 25, 45 and 85 cents, respectively. For sodas costing 6.94 cents, a 15-cent selling price was suggested. Dealers were cautioned to price correctly and to watch for consumer reaction to selling price as a guide to greater sales.

W. H. MARTIN

357. Price trends. ANONYMOUS. Ice Cream Trade J., 44, 6: 44, 45, 93-98. June, 1948.

Higher cost of dairy products has caused ice cream manufacturers to increase the wholesale price of bulk ice cream 10 to 15 cents per gallon and the price of packaged ice cream 15 to 25 cents per gallon. In the East, bulk vanilla ice cream is priced at \$1.60 to \$1.80 per gallon, with quantity discounts. In Chicago the price reported was \$1.85 to \$1.87 per gallon on bulk vanilla. Prices in the South, Oklahoma, California and Kansas range from \$1.40 to \$1.50 per gallon and in Minneapolis \$1.20 to \$1.30 per gallon.

W. H. MARTIN

MILK

358. Frozen storage of milk as a method of preservation. F. J. DOAN, The Pennsylvania State College. Milk Dealer, 37, 8: 44, 102-112. May, 1948.

A discussion of frozen concentrated skimmilk, frozen fluid whole milk

and frozen concentrated whole milk which the author summarizes as follows: Bulk frozen concentrated skimmilk is now being used, in a limited way, commercially for preserving milk solids-not-fat for later use, usually in ice cream. It has some advantages over other storage products and probably will be used more generally for this purpose. Another, frozen homogenized fluid milk, proved very satisfactory as a preserved substitute for fresh fluid milk for Army uses during the war where expense was not a limiting factor. It is unlikely, however, to become an important commercial article in the peace-time economy. The third, frozen concentrated whole milk can be employed for storing whole milk solids in bulk form and may at some future time appear on the retail market as one of the increasing number of frozen foods which can be held on hand in the home freezer or individual locker by the consumer. All three of these products are examples of an old method of frustrating nature's demolition corps, the micro-organisms, but they represent new foods protected by such means.

C. J. BABCOCK

359. Borden introduces oblong 2-quart glass bottle. ANONYMOUS. Milk Dealer, 37, 8: 43. May, 1948.

Introduction of the space saving oblong two-quart glass milk bottle at food stores in Chicago and vicinity is announced by Borden's Chicago Milk Division. Thus, Chicago becomes the second city in the country to have this type of bottle, as it was introduced in New York some time ago.

The oblong bottle takes up 28% less space in a home or store refrigerator than the round half-gallon bottle and takes 33% less space than 2 single round quart bottles. The oblong container takes up 328 cubic inches of space as compared with 456 cubic inches for the round bottle. Another advantage of the oblong bottle is that it is easier to pour from. The oblong shape permits a firm grip on the sides.

C. J. BABCOCK

360. Plant operation and efficiency. L. C. THOMSEN, University of Wisconsin. Milk Dealer, 37, 8: 47, 48, 140-148. May, 1948.

Under operating costs, it is pointed out that on the basis of best available averages it appears that presently most fluid milk plants will require 36 cents to 45 cents of every sales dollar for operation, reserves for depreciation and a reasonable return on a justifiable investment. Of this amount, well over one-half is paid out directly for wages and salaries. Executives with officer titles receive less than one-half cent of the sales dollar. Under the heading "Labor Savings" filling is pointed out as the key operation, and if it is not properly timed as to output, efficiency is inclined to suffer.

The following proposals for sales economies are given: (a) increase size of retail and wholesale loads by use of lighter containers and cases; (b) use

public utility types of practices to restrict areas of operation; (c) expand store sales, which have been shown to cut sales costs to less than one-half of those for retail delivery; (d) establish numerous milk or dairy stores; (e) still further curtailing services to the consumer by, for example, delivery every third day; and (f) offer quantity discounts.

In discussing ways of reducing milk losses, the author shows that total "paper" losses in ideally operating milk plants selling fluid milk and cream amount to approximately 1.65%, of which slightly less than three-fourths can be assigned to the fluid milk part of the operations, and slightly over one-fourth can be charged to the fluid cream part of the operation.

H.T.S.T. systems, proper routing of milk, methods of reducing waste and care of equipment are also discussed. C. J. BABCOCK

361. New products developments leading to greater milk sales. K. G. WECKEL, University of Wisconsin, Madison. Milk Dealer, 37, 8: 50-56. May, 1948.

Preventing oxidized flavor, unsterilized evaporated milk, uniform equipment, extended delivery routes and electronic temperature recorders are discussed as potentialities that can improve and affect the sales and distribution of market milk. C. J. BABCOCK

362. The manufacture of a high quality chocolate milk drink. BENJAMIN P. FORBES, Benjamin P. Forbes Co., Cleveland, Ohio. Southern Dairy Products J., 44, 1: 42-3. July, 1948.

Due to its greater miscibility, cocoa powder rather than chocolate is used in the preparation of chocolate milk drinks. The necessity for scrupulously observing the rules prescribed by the flavor manufacturer is obvious. In using reconstituted milks, the solids should be the same as in fresh milk.

Settling may be caused by: (a) insufficiently stabilized cocoa; (b) too much milk or insufficient butterfat content; (c) dilution of dairy drink by milk or water; (d) restriction of valves on pressure side of pumps, causing a homogenizing effect or excessive whipping by centrifugal pumps; (e) too acid milk (do not neutralize); (f) using soda-neutralized milk; (g) using frozen milk; (h) incorrect pasteurizing and cooling; (i) insufficient heat or too short holding time; (j) over-agitation during and after cooling; (k) powder not entirely incorporated; (l) not cooled to 40° or lower; (m) precooling below 140° in the vat.

Thickening may be caused by: (a) excessive pasteurizing temperature; (b) overstabilization; (c) use of lime or magnesium stabilizers; (d) improperly rinsed bottles; (e) milk which is too acid. F. W. BENNETT

363. Factors to consider in making high quality chocolate milk drinks.
W. C. THACKER. Southern Dairy Products J., 43, 2: 102-3, 116.
Feb., 1948.

Chocolate milk is not consumed as a substitute for white milk. In 3 urban markets, the total per capita consumption of milk was 18% higher in the families that included chocolate milk.

The 6 desirable characteristics of chocolate milk or chocolate drink are: (a) High quality milk or part-skimmed milk. The minimum fat standard for whole milk should be met. (b) Mild chocolate flavor. The amount of flavor used ranges from 1.0 to 1.5% cocoa or 1.5 to 2.25% liquor chocolate. Cocoa is most used. Quality varies widely. Prepared syrups are convenient for small distributors, but the addition of the dry ingredients before pasteurization is less expensive. (c) Little or no sedimentation with a low to medium viscosity. Special finely-ground cocoas are on the market. Sodium alginate will prevent sedimentation at a low viscosity. Homogenization increases sedimentation. (d) Elimination of the ragged dark-colored cream layer. This can be prevented by pasteurization at 160 to 165° F. for 15 to 30 minutes and the addition of stabilizers. (e) Light to medium red-brown color. (f) Medium to high sweetness. Consumer demands differ from about 5 to 8% sugar. Standard composition and processing methods are important.

F. W. BENNETT

364. Production can be leveled. H. F. SIMMONS, Michigan Milk Producers' Association. Milk Dealer, 37, 8: 92-100. May, 1948.

A discussion is given of the "Base and Surplus Plan" as a means of leveling the production of milk by farmers so that it more nearly conforms to fluid milk requirements.

C. J. BABCOCK

SANITATION AND CLEANSING

365. New sanitary practices and controls in the dairy industry. M. G. PEDERSON, Price's Creameries, Inc., El Paso, Texas. Southern Dairy Products J., 42, 1: 34-5, 42-3. July, 1947.

Sediment may be received in the ice cream plant through milk and its products, sweetening agents, stabilizers and even the city water. In the plant, sediment may be introduced from solid flavoring materials, air in the plant, undissolved particles of cleaning and sterilizing agents, lubricants not held back by packing glands and cartons which have not been fully protected. Unsanitary practices of employees are another factor. Insects and rodents are important. Applying a 5% or stronger emulsion of DDT to the walls and ceiling every 2 or 3 weeks should control flies. Sodium fluoride, pyrethrum, rotenone and roach poisons, when regularly and prop-

erly used, are effective against cockroaches. Daily cleaning of the plant and the elimination of cracks and unused equipment which may harbor them are essential. Mouse-proof construction, trapping, poisoning and the elimination of rubbish are means of exterminating mice and rats. Antu, thallium sulfate, barium carbonate, zinc phosphide and strychnine should be used by an experienced and intelligent person. F. W. BENNETT

366. Cleaning and disinfecting milk and ice cream processing equipment. M. A. BAILEY, The Diversey Corporation, Chicago, Ill. Southern Dairy Products J., 41, 4: 73, 78-81. Apr., 1947.

Clean-up operations consume over one-third of the labor in the dairy and therefore are worthy of serious consideration. In the 1920's, soap and washing soda were the two commonly used cleaning agents. Soap left a film of soap or scum on the equipment, did not possess the necessary cleaning power and was expensive to use. Action on the hands accompanied the use of washing soda, lime films were left and its cleaning performance was still far from efficient. Chemists set out to blend the various sodas with trisodium phosphate and metasilicate to obtain balanced cleaners.

In selecting the product to be used for general cleaning, the cleaner must remove contamination quickly and safely; no dirt or deposit must be left on the equipment; the cleaner must not damage the equipment; the cleaner must not be hard on the hands of the operator and must be liked by him; it must be economical to use judged by the results obtained.

There are six essential characteristics that a good cleaning compound should exhibit: water softening to eliminate a mineral build-up on the equipment; wetting action to penetrate and wet the film to be removed; suspending action to prevent the settling out of soil to re-adhere to the equipment; emulsifying ability to keep fats suspended and allow them to be rinsed off easily; rinsing ability so that the cleaner itself may be easily removed; bactericidal action in special operation such as bottle washing.

Milkstone may be removed safely and prevented by the successive use of specially prepared acid and alkaline cleaners in short-time pasteurizers, vacuum pans and evaporators.

Training of the workers in the care and cleaning of equipment is of the utmost importance. The importance of their work must be impressed upon them for best results. Trained representatives of several chemical companies will assist gladly in an educational plan for plant employees.

F. W. BENNETT

MISCELLANEOUS

367. Refrigeration for dairy plants. A. A. GEIGER, York Corporation, Atlanta, Ga. Southern Dairy Products J., 39, 5: 82, 88-9. May, 1946.

The refrigeration for dairy plants should be designed to handle easily

the maximum load, require no hand regulation of control valves for minimum loads and have sufficient flexibility. All efforts should be made to make the plant free of shut-down possibilities. There are 3 types of refrigerating mediums for cooling milk products: brine, direct expansion, or sweet water. For the last 10 years, milk machinery manufacturers of vertical vats with spray jackets have tended to recommend cooling by sweet water rather than brine. If an open type sweet water tank is used, an ice field is built up during the night to serve as a storage of refrigeration. As the ice melts during milk cooling, the temperature of the sweet water reaching the milk cooler may increase 20°. For this reason, this system cannot be recommended for milk plant use. The shell and tube water cooler to deliver 34° F. water is subject to freezing up. Instantaneous water coolers with controls to deliver water at uniform optimum temperature are available. Brine for cooling makes it possible to use a smaller milk cooler because of a lower brine temperature. When brine is used, only one ammonia control is necessary. The compressor in a brine system can be operated at 100% capacity. The wide range of temperature possible in the brine enables the plant to have a low enough temperature at all times.

Corrosion should not be a problem if the brine is not allowed to become acid. Brine may be used in a plate cooler if the tightening head is equipped with a compensating spring and a proper brine control is used. The direct expansion system is not advisable for small plants because of (a) excessive cost on small volumes, (b) complicated controls and (c) short-cycling of compressors. This system adapts itself advantageously in larger plants, but for buttermilk and cream cooling, the sweet water system is used.

A two-compressor installation is recommended rather than a single compressor for flexibility. Too few operators of milk plants devote enough attention to their refrigeration needs.

F. W. BENNETT

368. How employer-employee relationships reflect good management.
J. W. Post, Armour and Co., Chicago, Ill. *Ice Cream Rev.*, 31, 11:
76, 78, 80, 82, 84. June, 1948.

The losses or gains as a result of customer satisfaction or dissatisfaction depend upon the kind of personnel developed by the company. Management must assume the responsibility for seeing that the right kind of personnel is developed, as only a few good employees are self-made. Fourteen groups of desirable traits and habits which should be developed in employees are enumerated.

Better employer-employee relationship may be developed in many ways. Some of the methods currently used by well-managed businesses include the following: employee publications to improve the line of communications between employees and the company; job evaluation as it applies to wage

rates to prevent inequities; polling of employees for opinions, ideas and suggestions for improvement; regular training and educational procedure which is oral, visual and written; aptitude and psychological testing that help in employee selection and in job assignments; periodic merit rating by procedures which meet the need for individual appraisal; provision for recreational and health protection facilities; and posting of employment policies.

Another factor to be considered is that of federal and state legislation as it affects employer-employee relationships. Plans must be formulated to meet such things as sick-benefit plans, life, health and accident plans, profit-sharing or merit-rating plans. The position of management with respect to labor relations is entirely different from that of a few years ago. Business now is confronted with inflation, full employment and much new legislation which affects employer-employee relations.

Management must devote more time to the personnel problem. What your employees think and express determines in a large measure the public relations of the company concerned. How well the employer-employee relationship problem is solved will determine in no small measure the success of a given business under present-day conditions. W. J. CAULFIELD

369. Economic aspects of open and closed cream markets. LELAND SPENCER, Cornell University. Milk Dealer, 37, 8: 86-88. May, 1948.

Following a listing of the consequences of maintaining strict requirements as to conditions under which cream is produced and handled and also limiting the area of inspection, it is concluded that: (a) the "closed" market policy for cream does not result in higher cost to consumers for fluid milk; (b) the extra income for producers is obtained through higher prices for cream than would prevail in an "open" market; (c) the extra cost of cream is shared by consumers and distributors of fluid cream and sour cream and by manufacturers of ice cream.

Another point worthy of emphasis is that the milk produced for use as cream in a "closed" market is a reserve supply that can be drawn upon for use as fluid milk whenever the milk supply from inspected sources is insufficient for all demands.

The advantages and disadvantages of requiring cream to be produced and handled under strict sanitary requirements within a limited production area are difficult to evaluate. Considerations of public health and aesthetics as well as economics are involved. There is need for a thorough and careful determination and appraisal of pertinent facts as a basis for intelligent action.

C. J. BABCOCK

370. Trend toward conversion to oil as fuel continues. ANONYMOUS.
Milk Dealer, 37, 8: 40. May, 1948.

A survey made by the Olsen Publishing Co. of milk dealers in New England and North Central states shows that the trend among milk dealers toward converting plants from coal to oil is continuing.

Out of 434 replies received to questionnaires, 54.5% reported they are now using oil as fuel, 43.4% are using coal, and 3.1% are using gas. Among these same dealers, however, 14.1% indicated they are planning to make a change in the type of fuel used, and of these, 71.5% expect to change from coal to oil and another 10.7% from coal to gas.

The determining factor mentioned most often by respondents to the questionnaire was that oil or gas was cleaner than coal. Second most popular determining factor was that oil or gas saves labor; third was convenience; fourth was price; and fifth was efficiency. C. J. BABCOCK

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ABSTRACTS OF LITERATURE

BOOK REVIEWS

371. *Proteins and amino acids in nutrition*. M. SAHYUN, editor. 566 pp. \$7.50. Reinhold Publishing Corporation, New York, N. Y., 1948.

Eighteen eminent authorities associated with various fields of nutrition have contributed to this volume, which contains a wealth of information relating to the nutritional role of proteins and amino acids. The first chapter is devoted largely to a historical review of research related to the subject. A number of subsequent chapters consider the general aspects of the role of protein in nutrition, whereas others deal with specialized subjects such as hormones, plasma proteins, toxins and filterable viruses. Although the subject matter is treated primarily from the standpoint of human nutrition, one chapter is devoted to the amino acid requirements of the avian species, and frequent reference is made to other species, especially the bovine. A comprehensive list of literature references is presented at the end of each chapter and an extensive index covers the subject matter of the entire book. The appendix contains two tables which indicate the nutritive value of a large variety of human foods.

This book should be of interest to all who are concerned with the fields of either human or animal nutrition.

N. L. Jacobson

BREEDING

372. *Hereditary epithelial defects in ayrshire cattle*. F. B. HUTT AND J. N. FROST, Cornell University. *J. Heredity*, 39: 131-137. 1948.

An epithelial defect, less severe but otherwise similar to that found in Holstein-Fresian cattle by Hadley and Cole, was found in 4 registered Ayrshire calves. Raw areas were found in knees and distal extremities of all four limbs just above the hoofs and, to a lesser degree, on the muzzle. Mucosa was lacking under the tongue in one calf examined. Another calf had lesions on the inner surface of the ears. Three calves were vealed at 2-3 weeks. One calf that was protected against bacterial invasion by sulphathaladine prophylaxis was destroyed at 3.5 months. Breed difference was suggested as being due either to genotype in which the mutant operates or, in the case of Jerseys, to a different gene. The defect apparently was caused by a single autosomal recessive gene carried by the sires, Whitpain Skytop and Mansfield May's Augie.

L. O. Gilmore

CHEMISTRY

373. The glyceride composition of milk fat. C. P. ANANTAKRISHNAN, V. R. BHALERAO, AND T. M. PAUL, Indian Dairy Research Institute, Bangalore, India. *Arch. Biochem.*, **18**, 1: 35-40. July, 1948.

Sindhi cows were fed a basal ration supplemented with cottonseed, sesame and hydrogenated cocoanut and groundnut oils at the rate of 1.5 lb. per head per day. In comparison with the butter fat of the control group, reduced Reichert values and increased iodine values, butyrorefractometer readings and saponification values were observed. With the exception of the group fed hydrogenated cocoanut oil, a reduction in the total amount of acids up to C_{14} occurred. Supplements of cottonseed, sesame and hydrogenated groundnut oils led to an increase in the oleoglycerides of the milk fat. Rations with a high percentage of linoleic acid failed to show increased amounts of this constituent in the milk fat.

H. J. Peppler

374. A browning reaction involving copper-proteins. J. B. THOMPSON, R. B. KOCHER, AND H. W. FRITZSCHE, Quartermaster Food and Container Institute for the Armed Forces, Chicago, Ill. *Arch. Biochem.*, **18**, 1: 41-50. July, 1948.

A browning reaction produced by copper-protein complexes is described. The mechanism is different from known types of enzymatic browning. Reactions of the copper-proteins of casein, gelatin and albumin with compounds containing an ethylene group within a ring structure are discussed. Tryptophane, cystine and an acid hydrolyzate of casein did not replace casein in the browning reactions, thus indicating that copper was bound through forces existent in polypeptides rather than amino acids.

H. J. Peppler

CONCENTRATED AND DRY MILKS; BY-PRODUCTS

375. Process for manufacture of milk sugar. D. D. PEBBLES AND T. V. MARQUIS. (assigned to Western Condensing Co.) U. S. Patent 2,439,612. 8 claims. April 13, 1948. *Official Gaz. U. S. Pat. Office*, 609 (2): 400. 1948.

Whey first is condensed after receiving sufficient heat by direct steam to coagulate the protein. The condensed product, supersaturated to lactose and free of lactose crystals then is heated by direct steam, cooled by evaporation and seeded with lactose. After mass crystallization, the lactose is removed by centrifugal force, washed and dried.

R. Whitaker

DISEASES

376. Some observations on postparturient cows in four separate herds as related to expulsion of their fetal membranes. W. L. BOYD AND A. F. SELLERS. *Cornell Vet.*, **38**, 3: 263-266. July, 1948.

Data from 1 Bang's-positive and 3 Bang's-negative herds are presented in

relation to expulsion of fetal membranes. In a series of 542 parturitions, rates of placental retention appeared high in cases of abortion and stillbirth regardless of Bang's status, but the rate of retention was approximately 3 times higher in the Bang's-positive herd than in the Bang's-negative herds, in the viable, single-calf group. It also was observed that retention rates were higher in viable, single-calf gestations terminating outside of the normal range of 274-291 days than in those terminating within normal range.

T. M. Ludwick

FEEDS AND FEEDING

377. All-year pasturing with and without concentrates for dairy cows.
B. P. HAZLEWOOD, Univ. of Tennessee, Knoxville. Tenn. Agr. Expt. Sta.
Bull. 207. 1948.

Two groups of 16 Jerseys were used in experiment. One group received no grain, the other group received grain at the rate of 1 lb. to each 3 lb. milk produced. Each group received all-year pasture, alfalfa hay and silage. Individual weights, production and feed-consumption records were kept. The experiment ran over a period required for the cows in each group to complete 2 successive lactations. Production of cows in the no-grain group was 76% of that of grain-fed group. Each group followed the same seasonal trend of production, but the grain-fed cows were the more persistent.

W. Dudley

378. Mineral metabolism studies in dairy cattle. IV. Effects of mineral supplementation of the prepartal diet upon the composition of the blood of cows and their calves at parturition. J. T. REID, G. M. WARD, AND R. L. SALISBURY. N. J. Agr. Expt. Sta., Sussex. J. Nutrition, 36, 1: 75-89. July 10, 1948.

Four groups of cows were fed a basal concentrate ration containing 1% calcium plus hay, silage and pasture in season during at least the last 2 months of gestation. Group I received the basal concentrate, group II the basal concentrate plus 2.5% calcium carbonate, group III basal concentrate plus 3% calcite flour and group IV basal concentrate plus 3% mico. The supplement for groups III and IV contained various trace elements. Blood or plasma analyses were made for 49 dams and 50 calves just following parturition. Only 7 of the calves received colostrum. These supplements had no effect upon levels of calcium, phosphorus and several other blood constituents. However, both cows and calves of group III had lower levels of reduced and total glutathione than other groups. Calves blood contained greater concentrations of reduced and total glutathione, calcium, inorganic phosphorus, acid and alkaline phosphatase and ascorbic acid and greater numbers of erythrocytes than their dams. The dams had greater corpuscular hemoglobin content and corpuscular volume and higher levels of total proteins, globulin and albumin. Sex had no effect on constituents of blood of calves.

R. K. Waugh

HERD MANAGEMENT

379. Efficient mechanical milking. W. G. WHITTLESTON. Australian J. Dairy Technol., 3, 2: 45-72. 1948.

This article reviews 3 aspects of the problem—the influence of various factors on the process of getting milk from the cow, the construction of the milking machine and the installation, care and servicing of the machine. The discussion is of such length that detailed abstracting is not practical. The author approaches his subject in a thoroughly critical manner; he is an advocate of simplification of the machine and of its use.

F. E. Nelson

ICE CREAM

380. Foreign fats in ice cream. ANONYMOUS. Ice Cream Trade J., 44, 7: 34. July, 1948.

The Texas State Board of Health has ruled that all ice cream shall contain 8% butterfat to which may be added 4% vegetable fat. Prior to this ruling, all state laws and regulations have clearly specified that the fat content of ice cream must come from dairy products except for the fats present in such items as chocolate, but even in such cases, only a reasonable tolerance has been allowed. The International Association of Ice Cream Manufacturers and the Texas Dairy Products Institute are trying to get the ruling changed.

W. H. Martin

381. Ice cream package for the elite. ANONYMOUS. Ice Cream Trade J., 44, 7: 32. July, 1948.

A new product in a transparent plastic container, representing a radical departure in ice cream package merchandising, has been introduced in the New York market by the Rosemarie de Paris, one of the country's most distinctive chocolate and confectionary firms. The ice cream, a French type containing 18% butterfat and low in overrun, is made in 5 basic flavors—vanilla, chocolate, coffee, strawberry and pistachio. Pints retail for 85 cents and quarts for \$1.60.

The product is packaged in a new transparent acetate tub-shaped container, measuring 3 inches in diameter at the bottom, 3 $\frac{3}{4}$ inches at the top and about 4 inches high. The lid is a disk-shaped paperboard. The famous Rosemarie de Paris coach, the company's trade mark, is reproduced on the package but does not hide the contents which are plainly visible.

W. H. Martin

382. Carton for ice cream and the like. S. H. BERCH. U. S. Patent 2,443,530. 4 claims. U. S. Patent 2,443,531. 2 claims. June 15, 1948. Official Gaz. U. S. Pat. Office, 611 (3): 721. 1948.

A container for ice cream and other frozen foods is described which is cut from 1 piece of material and scored in such a manner that it may be folded to produce a carton held in shape by interlocking flaps and requiring no adhesive.

R. Whitaker

383. Brine tank. J. H. REAGIN. U. S. Patent 2,442,146. 3 claims. May 25, 1948. Official Gaz. U. S. Pat. Office, 610 (4) : 942. 1948.

The distinguishing feature of this brine tank for making frozen confections is its shape. The tank is round, the product freezing in the molds during one revolution around the tank. Cold brine is supplied continuously from a supply tank equipped with refrigeration coils.

R. Whitaker

384. The retail store. D. GHORMLEY. Ice Cream Trade J., 44, 7: 40. July, 1948.

There seems to be good economic justification for the specialized ice cream store. These stores can give the best of service; the stores are built and equipped and the personnel are trained to eliminate problems commonly faced by customers in search of ice cream. The stores are conveniently located, they sell for cash and they can sell for less and make a profit. There are some disadvantages to this type of merchandising. The season is short, the hours are long, the product is perishable and competition plentiful. The ice cream merchant must weigh these advantages and disadvantages and then determine his course of action.

W. H. Martin

MILK

385. Heat exchanger. F. P. HANRAHAN. (Assigned to people of the U. S.) U. S. Patent 2,445,115. 2 claims. July 13, 1948. Official Gaz. U. S. Pat. Office, 612 (2) : 422. 1948.

In a device for heating milk and other fluids and having such desirable features as sanitary construction, rapid continuous heating and compact size, the milk is caused to flow at high velocity in a spiral channel between two heating surfaces. Only two relatively small replaceable gaskets are employed.

R. Whitaker

386. Milk bottle closure. F. M. ALEXANDER. U. S. Patent 2,442,745. 1 claim. June 8, 1948. Official Gaz. U. S. Pat. Office, 611 (2) : 410. 1948.

A closure for glass milk bottles is described consisting of a hinged disk which may be raised or lowered by pressing on a wire handle. The device is attached to the bottle neck by means of a spring jaw.

R. Whitaker

387. Cover and filter for milker pails. T. J. PFETCHER. U. S. Patent 2,445,122. 1 claim. July 13, 1948. Official Gaz. U. S. Pat. Office, 612 (2) : 444. 1948.

A combination cover and filter for milk pails is described. R. Whitaker

SANITATION AND CLEANSING

388. Sterilizing arrangement for milk tubes. J. L. FAIR. U. S. Patent 2,441,878. 3 claims. May 18, 1948. Official Gaz. U. S. Pat. Office, 610 (3) : 710. 1948.

A device is described for maintaining the rubber teat cups of milking machines full of a sterilizing solution when not in use. R. Whitaker

389. Apparatus for washing milking machines. R. C. HERMAN. U. S. Patent 2,442,926. 1 claim. June 8, 1948. Official Gaz. U. S. Pat. Office, 611 (2) : 454. 1948.

A tank containing water and under air pressure is situated under a basin for cleaning milking machine parts. From the tank, air and water lines, equipped with valves, feed a nozzle over the basin. R. Whitaker

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ABSTRACTS OF LITERATURE

BOOK REVIEWS

390. The artificial insemination of dairy cattle. A handbook and laboratory manual. H. A. HERMAN AND F. W. MADDEN. 95 pp. Lucas Bros., Columbia, Mo. 1947.

This manual is designed especially for use in teaching laboratory work in courses dealing with the artificial insemination of dairy cattle. It also should be of interest and value to managers, technicians and field workers in artificial breeding associations, as well as others interested in the technics employed.

Twelve of the 20 exercises deal with the collection, examination, dilution, storage and transportation of bull semen and insemination of the cow. Particular emphasis is placed on laboratory methods for evaluating the quality of semen. Brief illustrated discussions of the anatomy and physiology of the reproductive organs of the bull and cow are presented, along with exercises covering pregnancy determination and reproductive troubles of dairy cows. In addition to reviewing some of the factors involved in the selection, management and care of sires, there are exercises pertaining to the organization of artificial breeding associations and record keeping.

The questions and references found at the end of each exercise should be of special aid to the student in comprehending and enlarging upon the principal points presented. For teaching at the college level, the authors suggest that this handbook be supplemented liberally with more detailed lecture material.

J. O. Almquist

391. Standard methods for the examination of dairy products. 9th Edn. \$4.00. American Public Health Association, 1790 Broadway, New York 19, N. Y. 1948.

This long-awaited new edition of the standard publication in this field constitutes a distinct departure in the presentation of the material covered. Because of the expanded coverage, cross references have become essential to avoid much undesirable duplication. The separation of discussion of application from the actual directions for the test in question should simplify use of the book by the various interested groups. Drs. Robertson and Black are to be commended for their editorial work, for they have integrated the material on the various tests in a manner which makes a very definite contribution to the usefulness of the publication.

Only a few of the specific changes will be enumerated. Incubation of plates

for plate counts now is specified at either 32 or 35° C. (No indications of tolerances allowable are given.) The 1-hour and triple-reading procedures for the resazurin test are recognized. Hourly inversion of both methylene blue and resazurin reduction tests becomes the standard procedure. The section on sediment testing has been enlarged considerably. Laboratory procedures for the phosphatase test are recognized in the main body of the publication, and field tests are incorporated in the chapter on "Screening Tests". In this chapter also are included many tests or modifications of tests which will serve for routine control but which are considered unsuitable for official or regulatory action. In addition to methods for assay for vitamin D, methods for thiamine, riboflavin and niacin are given, being quoted from the twelfth revision of the *United States Pharmacopoeia* and the second bound supplement thereof.

This ninth edition of *Standard Methods* is a volume which should be consulted repeatedly in every dairy control laboratory in the country and should be read, at least in part, by all who have occasion to use or interpret the results of laboratory control of dairy products in manufacturing or merchandising activities.

F. E. Nelson

BACTERIOLOGY

392. Newly proposed staining formulas for the direct microscopic examination of milk. B. S. LEVINE AND L. A. BLACK, U. S. Public Health Service, Cincinnati, Ohio. *Am. J. Pub. Health*, **38**, 9: 1210-1218. Sept., 1948.

A two-dip staining procedure was adopted for study using 0.6% methylene blue in 95% ethyl alcohol to stain the prepared smears after defatting. Also a single dip stain, free from water and acid was prepared as follows: 0.6 g. certified methylene blue powder, 50 ml. of 95% ethyl alcohol and 50 ml. of technical grade tetrachlorethane. A comparison of the carbolated methylene blue stain, Newman-Lampert stain and the water- and acid-free stain proposed by the authors showed that bacteria and leucocyte counts of raw milk obtained by the carbolated methylene blue and the Newman-Lampert stain agreed closely. However, in the smears stained by the authors' procedure, the bacteria count averaged 94% higher and the leucocyte count averaged 9% below either of the other two methods used. The use of a polychrome methylene blue stain resulted in counts of both bacteria and leucocytes higher than when the carbolated methylene blue stain was used, but lower than those obtained with the acid- and water-free stain. No polychrome effects were noticed in the raw milk smears. The data obtained indicate that the water- and acid-free stain will yield maximal counts.

D. D. Deane

393. The bacteriological examination of ice cream. Part 2. JOYCE CRANFIELD, Edinburgh University. *Dairy Inds.*, **8**, 8: 800-804. Aug., 1948.

One hundred samples of ice cream were tested by a modified methylene blue test with a pre-incubation period of 17 hours at atmospheric shade temperature below 20° C. Ninety per cent of samples with plate counts of more than 200,000

organisms per ml. and *E. coli* present in 0.01 ml. were graded as 3 or 4 by the methylene blue reduction test. One hundred per cent of samples with plate counts of less than 200,000 organisms per ml. were graded 1 or 2 by the methylene blue test.

Results are given on 20 samples of ice cream to show the change in the total count and *E. coli* content after (a) refrigeration overnight and (b) standing at atmospheric temperature overnight. Overnight refrigeration tends to bring about a slight increase in plate count but a decrease in the coliform content. In most cases, the correlation between the plate count and coliform content and the grading of the samples according to the methylene blue test was unchanged by overnight refrigeration. The plate count always increased and the coliform content frequently increased when samples were held at room temperatures overnight. Little correlation was found between the results of the tests carried out on the second day and the grading of the samples according to the methylene blue test on samples held overnight at room temperature.

G. H. Watrous

394. The resazurin test—a review of the literature from 1940–1948. D. W. WATSON. Dairy Inds., 8, 8: 751–762. Aug., 1948.

A fairly complete collection of the most important papers from 1940 to 1948 is presented under the headings: (a) Synthesis and standardization of the dye; (b) Technic; (c) Fundamental work; (d) Mastitis and abnormal milk; and (e) Modifications, practical applications, and comparisons with other tests.

G. H. Watrous

395. Esters of vanillic acid as spore-controlling agents. F. R. EVANS AND H. R. CURRAN. Bureau of Dairy Industry, U.S.D.A., Washington, D.C. Food Research, 13, 1: 66. Jan.–Feb., 1948.

The spore-controlling action of 4 vanillic acid esters in milk at 0.10 and 0.15% concentrations was studied over periods of 3 to 4 months against cultures comprising 20 mesophilic aerobes, 10 thermophilic aerobes, and four mesophilic anaerobes, temperature of storage being 20, 37 or 52° C.

Isobutyl vanillate was the most efficient preservative. In 0.15% concentration, all samples remained unchanged over the storage period. N-butyl vanillate was slightly less effective, particularly against anaerobes. Ethyl vanillate delayed but seldom prevented growth. Sodium benzoate in 0.15% concentration in milk was relatively ineffective as a spore-inhibiting agent but had little effect on flavor. However, a like amount of the vanillates produced a distinctly objectionable flavor. The action of the reagents was judged to be primarily sporistatic.

F. J. Doan

396. A new and rapid method for the preparation and standardization of Brucella Ring Test antigen. R. M. Wood, Dept. of Bact., Johns Hopkins School of Medicine, Baltimore, Md. Am. J. Pub. Health, 38, 9: 1225–1227. Sept., 1948.

The author proposes a new and more rapid method of preparing the antigen

used in the Ring test for *Brucella* agglutinins in milk. A smooth strain of *Brucella abortus* is grown in broth for 24-48 hours to obtain massive growth, harvested, treated with phenol and held at 60° C. for 1 hour, then chilled, centrifuged and the supernatant discarded. After resuspension in distilled water, the cells are filtered through glass wool, washed twice more and then resuspended in 2 volumes of distilled water. The suspended cells are then stained with hematoxylin, centrifuged down and washed with distilled water until the supernatant is colorless. After resuspending in 0.85% saline containing 0.5% phenol, the stained antigen is standardized and checked for sensitivity. Substitution of the stained *Brucella* antigen for the unstained antigen normally used in either the slow tube or rapid plate method of serum agglutination results in an endpoint that is seen more easily. The author found the Ring test method, using the stained antigen as described, was fully as sensitive and had fewer sources of error than the whey titration for detecting *Brucella* agglutinins in milk. D. D. Deane

397. The bacterial activity of "racemized casein", caseose, and the four diastereoisomeric leucylleucines. S. W. FOX, Y. KOBAYASHI, S. MELVIN, AND F. N. MINARD, Chem. Lab., Ia. State College, Ames. J. Am. Chem. Soc. 70, 7: 2404-2406. 1948.

Experiments with racemized casein and caseose indicate that a polypeptide preparation containing a larger proportion of D-amino acid residues than occurs in tyrocidine is without appreciable antibacterial activity when tested against *Escherichia coli* or *Lactobacillus arabinosus* 17-5. H. J. Peppler

398. Pasteurization of liquid-egg products. IV. Destruction of coliforms. A. R. WINTER AND G. F. STEWART, Ia. Agr. Expt. Sta., Ames, AND MARIAN WILKIN, Ohio State Research Foundation, Columbus. Food Research, 13, 1: 11. Jan.-Feb., 1948.

Coliform bacteria were found in all (134) samples of commercial liquid, frozen and defrosted whole egg. Those found in unfrozen, liquid whole egg samples were destroyed more easily by pasteurization than those found in defrosted liquid whole egg samples. Pasteurization of unfrozen and defrosted liquid whole egg samples at 146° F. for 0.5 minute, 144° F. for 1.0 minute, 142° F. for 1.5 minutes and 140° F. for 2.5 minutes totally destroyed the coliform bacteria in all but one of the samples. F. J. Doan

CHEESE

399. Composition control of cheddar cheese. H. L. WILSON, Kraft Foods Co., Chicago, Ill. Southern Dairy Products J., 44, 2: 30-42. Aug., 1948.

The following conditions tend to increase the moisture content and the opposite conditions tend to decrease it: less starter, more rennet, cutting curd as firm as possible, lower cooking temperature within a range of 98-102° F., less agitation, pushing back curd sooner before draining, packing curd deeper, cutting into wider

slabs, piling sooner, faster and higher, lower temperature during cheddaring, shorter cheddaring time, milling into larger pieces, shorter periods of forking after milling and salting, shorter holding period after salting and the use of larger size hoops.

Lower moisture content of the curd and the addition of the salt more slowly and in more applications tend to increase the salt content. The uniformity of salt content is increased by uniform distribution of salt, uniform depth of curd in vat, forking curd from ends and sides into the center of vat, and ditching the curd to allow the whey to run off. Piling the curd and allowing it to stand as long as possible, but still be able to free it of lumps, will tend to eliminate mechanical holes. Alternate piling and forking must be repeated until the salt is thoroughly dissolved.

F. W. Bennett

400. Brown discoloration in malted process cheese. I. HLYNKA AND E. G. HOOD. Division of Chemistry and Division of Bacteriology and Dairy Research, Dept. of Agriculture, Ottawa, Canada. Food Research, 13, 3: 213. May-June, 1948.

Experiments with malted cheese (a blend of process cheese with malt syrup) revealed that the browning defect to which this cheese is subject is caused by an interaction of amino acids and proteins of the cheese with aldose sugars in the malt and results in an accompanying increase in titratable acidity and lowering of pH. It was concluded that malted cheese can be protected from this color defect by additions of sulfites or sulfur dioxide, the use of which is regulated in foods by the various departments of health and/or foods chemistry.

F. J. Doan

401. Cottage cheese. G. C. NORTH, AND L. LITTLE. (Assigned to Beatrice Creamery Co.). U. S. Patent 2,446,550. 3 claims. Aug. 10, 1948. Official Gaz. U. S. Pat. Office, 613 (2), 364. 1948.

Cottage cheese is prepared by cutting enzyme coagulated curd at a whey acidity of between 0.25 and 0.45%, cooking to obtain a rubbery tough body, freezing the curd and then thawing to produce a firm bodied and smooth textured product.

R. Whitaker

CHEMISTRY

402. Vitamin A content of Cuban Cow's milk and of liver oils of Cuban sea sharks. J. J. ANGULO, R. F. COWLEY, ALBERTO MAIRERO, AND CESAR FUENTES, School of Medicine, Havana University and Laboratory of Compania de Pesca del Valle, S. A., Havana, Cuba. Food Research, 13, 1: 1. Jan.-Feb., 1948.

Fifteen samples of cow's milk obtained from Havana cafeterias during the late summer and autumn indicated that boiled milk contained the greatest quantity of vitamin A and carotene, followed in order by Grade A milk (raw or pasteurized). The higher levels encountered in the boiled milk were attributed to the loss of water as a result of boiling. The levels of vitamin A and carotene

in Cuban Grade A milk were higher than values in the literature for Guernsey milk and considerably higher than for Holstein, Brown Swiss and Jersey milk.

F. J. Doan

403. The specific refractive increment of some purified proteins. G. E. PERLMANN, AND L. G. LONGSWORTH. Rockefeller Institute for Medical Research. J. Am. Chem. Soc., 70, 8: 2719-2724. 1948.

Data were obtained for the quantitative interpretation of the electrophoretic patterns of bovine serum, beta lactoglobulin, egg albumin, human serum and human gamma globulin. By means of a hollow, prismatic cell and the optical equipment of the Tiselius electrophoresis apparatus, the refractive index increments of solutions of the purified proteins were measured as a function of protein concentration, temperature and wavelength of incident light. The changes in specific refractive increment occurring on titration of the protein with alkali, in the presence of neutral salts and after equilibration with buffers, also were determined.

H. J. Peppler

DISEASES

404. Brucellosis in industry. C. F. JORDAN, Iowa State Dept. Health. Ind. Med., 17, 5: 176-180. May, 1948.

Consideration is given to the subject of brucellosis as related to industry. Discussion is based on epidemiologic investigation and on a series of 2,405 case reports assembled during the period, 1940-1946, through interest and courtesy of Iowa physicians. Disregarding 25% of the patients who gave no history of contact with animals, attention is directed to the larger number (75%) in Iowa who gave the history of direct contact with farm animals (hogs, cows, sheep, goats) prior to onset of symptoms. Illness in this group is shown to be closely associated with occupations including male farm workers, packing house employees, veterinarians, stock dealers and locker and rendering plant workers. Special consideration is given to the meat packing industry, to measures aimed at eradication of brucellosis in farm animals and at prevention of human illness. With cooperative effort on the part of all interested agencies and individuals, it is certain that continued application of control and preventive measures will reduce the vital hazards of direct contact with infected tissues in proportion as further progress is made toward eradication of brucellosis in farm animals.

Ruth E. L. Berggren

405. Brucellosis (undulant fever). G. R. TUREMAN, JR., 26 W. Williamsburg Road, Richmond, Virginia. Virginia Med. Monthly, 75, 1: 32-38. Jan., 1948.

From the work of many reliable investigators, brucellosis in man has been shown to be far more prevalent than previously conceived. It is not a self-limited disease running its course in a few days to a few months. Specific therapy, in the form of heat-killed organisms from abortus strains, offers the

greatest hope of cure or improvement in chronic cases. The disease should be considered in all cases of fever of undetermined origin and clinical pictures of a bizarre nature. In making a diagnosis, a combination of all the laboratory tests together with clinical findings should be utilized. The methods of prevention should be stressed, especially in those areas where the disease is commonly recognized. Cases of habitual abortion, in which other causes have been ruled out, should be investigated for brucellosis.

Ruth E. L. Berggren

ICE CREAM

406. Dealers profits in dipping bulk ice cream. V. M. RABUFFO. Ice Cream Trade J., 44, 8: 44-47, 96-100. Aug., 1948.

Tests conducted in New York City have shown that the average ice cream dealer, based on today's costs and selling prices, is making a gross profit of better than 40% without figuring overhead, refrigeration, insurance, etc. Mark-ups on bulk ice cream ranged from 25.2 to 160.4%. Three dealers had a gross of 60% or more, 2 had 50%, 15 had 40%, 15 had 30% and 5 had 20% or more.

Another survey in New York City showed that the average gross profit was 47.8%. The average contents of a 10-quart can was sold to consumers in the following form: 2.875 half pints; 3.5 pints; 0.925 quarts; 14.475 cones; 1.75 dishes; 5.125 sundaes; 13.2 sodas; 9.425 malted and some slight sales going into shakes, floats, banana splits, etc. The average retail selling prices were: half pints, 25 cents; pints, 45; quarts, 90; cones, 8; sundas, 25; dishes, 20; sodas, 20 and malted, 20 cents.

W. H. Martin

407. Ice cream cutting and discharging device. H. A. ALBERT. U. S. Patent 2,444,486. 8 claims. July 6, 1948. Official Gaz. U. S. Pat. Office, 612, (1): 153. 1948.

A scoop which delivers a half sphere of uncompressed ice cream is characterized by a rotating round bowl and a fixed perpendicular member for discharging the molded ice cream.

R. Whitaker

408. Milk products and the like containing algin compound. V. C. E. LEGLOAHEE. (Assigned to Algin Corp. of America). U. S. Patent 2,445,750. 3 claims. July 27, 1948. Official Gaz. U. S. Pat. Office 612 (4): 921. 1948.

When the acid radical of algin is partially satisfied with calcium and partially with an alkali metal, yielding a final pH of at least 7, it forms a desirable stabilizer for ice cream and other dairy products. The proportion of calcium, as metal, to algin is from 3.1 to 3.5% by weight.

R. Whitaker

PHYSIOLOGY

409. Penicillin blood and milk concentrations in the normal cow following parenteral administration. MARK WELSH, P. H. LANGER, R. L. BURK-

HART, AND C. R. SCHROEDER, Lederle Lab. Div., American Cyanamid Co., Pearl River, N. Y. *Science*, 108, 2799:185-187. August 20, 1948.

The purpose of this report is not to suggest the parenteral administration of penicillin for the treatment of mastitis. Penicillin can be localized in the udder by intramammary infusions without as rapid loss as when the drug is administered parenterally, and higher concentrations can be achieved on smaller dosage and less frequent administration. However, there is diffusion of penicillin from blood to milk. The total dosage of an antibiotic or a sulfonamide used during the course of treatment is relatively unimportant. However, the dose-time-weight relationship is important. Ruth E. L. Berggren

410. Physiological action of sodium carboxymethylcellulose on laboratory animals and humans. H. A. SHELANSKI AND A. M. CLARK, Smyth Laboratories, Philadelphia, Pa. *Food Research* 13, 1:29. Jan.-Feb., 1948.

This rather extensive investigation corroborates previous studies showing that sodium carboxymethyl cellulose (C.M.C.) is harmless physiologically when ingested, even in large amounts, by various animals and humans. These studies presented evidence indicating that "C.M.C." is not absorbed from the intestinal tract, but is almost quantitatively in the feces. F. J. Doan

MISCELLANEOUS

411. Supporting rack for milk strainers. H. E. MURDOCK. U. S. Patent 2,445,859. 2 claims. July 27, 1948. *Official Gaz. U. S. Pat. Office*, 612, (4):950. 1948.

A simple wire rack is described for holding a strainer in place in a separator bowl. R. Whitaker

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

BOOK REVIEWS

412. Annual review of biochemistry, Vol. 17. J. M. LUCK, editor. 801 pp. \$6.00. Annual Reviews, Inc., Stanford, Calif. 1948.

This volume constitutes a worthy successor to the preceding volumes in this series. As usual, the range of the material covered is extensive. The chapters are: Biological oxidations and reductions; Nonoxidative enzymes; chemistry of the carbohydrates; The chemistry of the immunopolysaccharides; X-ray crystallographic studies of compounds of biochemical interest; Chemistry of the lipids; The chemistry of the proteins and amino acids; Nucleoproteins, nucleic acids, and related substances; Carbohydrate metabolism; Lipid metabolism; The metabolism of proteins and amino acids; The metabolism of drugs and toxic substances; Clinical applications of biochemistry; Biochemistry of the hormones; The vitamins; Clinical aspects of vitamins; The biochemistry of carcinogenesis; Biochemistry of the natural pigments; The terpenes (in relation to the biology of genus pinus); The alkaloids; Photosynthesis; Mineral nutrition of plants; Plant hormones; Bacterial metabolism; The chemistry of penicillin; Ruminant digestion; Physiological aspects of genetics. Of these 27 chapters, 9 are written by scientists resident in foreign countries.

The large numbers of citations of original literature permit one to refer to the original articles reviewed with a minimum of difficulty. The indexing appears to be done well. The authors and editors are to be commended for making available such a satisfactory resumé of current biochemical literature.

F. E. Nelson

BACTERIOLOGY

413. Sur l'utilisation de le chloropicrine en laiterie (On the utilization of chloropicrin in the dairy). J. PIEN, Laiterie des Fermiers Reunis, Paris, France. Lait, 28, 271-272: 1-21. Jan.-Feb., 1948.

During 1944 it was proposed by Bertrand (Compt. rend., 219: 230. 1948) that in emergencies milk might be preserved by the addition of chloropicrin. The term microlysatation was proposed for the process, being derived from microlysine, a purified form of chloropicrin. The proposal received approval by the Academy of Medicine and by the Milk and Food Commission for temporary use. Pien reports on the practical aspects of experiments in which treated milk was placed on sale in Paris.

Chloropicrin is not readily soluble in milk and must be incorporated by vigorous agitation. In the trials conducted, concentrated lots were made up and

mixed with milk as received at country receiving stations. This milk, of poor sanitary quality and neither pasteurized nor refrigerated, was placed on sale in Paris. When treated at the rate of 50 mg. per l. the complaints were numerous and vehement due to its instability to boiling. When 80 mg. per l. was used, similar complaints were received and in addition the irritating odor and flavor, even after boiling, were noticed. Sales were discontinued after 12 d.

The rate at which acidities increased in treated milk was studied in the laboratory. Acidity changes were held in check for 2 d. at 25° C. but began to increase after 3-4 d. if the initial contamination was high. Similar results were obtained at 37° C. Upon heating, the milk separated into a smooth, fine curd and a clear upper layer. Total bacterial counts, 24 hr. after the addition of 50 mg./l. to poor quality milk, were large and consisted chiefly of lactic acid producers although large numbers of coliforms, putrifiers and spore-formers were present. The use of 80 mg./l. caused a gradual decline in numbers for 100 hr., while with only 50 mg./l., the numbers doubled in that length of time.

Additional experiments in which chloropierin was introduced to the milk immediately after milking resulted in good inhibition of bacterial growth. It is concluded, however, that practical difficulties make the use of this disinfectant impossible on the farm.

O. R. Irvine

414. Antibiotics active against bacterial viruses. I. N. ASHESHOV, FRIEDA STRELITZ, AND ELIZABETH HALL, Univ. of Western Ontario, London, Can. Can. J. Pub. Health, 39, 2: 75. Feb., 1948.

Paper discs soaked in substances active against bacteriophages were placed on agar plates flooded with bacteria and phage mixtures. Where the substances showed activity, the disc was surrounded by a zone of normal bacterial growth while the remaining parts of the plate would show confluent clearings of the phage.

An *Aspergillus* was found to produce 2 anti-phage substances, the first an anti-staphylophage substance, acidic in nature, stable between pH 2 and pH 10.0, which withstands heating to 100° C. It has been obtained in crystalline form. Less than 1 part in 10,000,000 by weight will inhibit staphylophage, while staphylococci require concentrations of 1 : 50,000 to 1 : 100,000 before being affected. The second substance from the same mold was active against 3 streptophages out of 12 tried. It is basic and considerably less stable than the anti-staphylophage substance.

O. R. Irvine

415. Method of preparing a vitamin concentrate. N. E. RODGERS, H. L. POLLARD, AND R. E. MEADE. (Assigned to Western Condensing Co.) U. S. Patent 2,449,144. 4 claims. Sept. 17, 1948. Official Gaz. U. S. Pat. Office, 614, 2: 434. 1948.

The fermentation of whey and skim milk with *Clostridium acetobutylicum* for the purpose of making a vitamin concentrate, including riboflavin, is improved through the use of a starter which has been transferred a sufficient number of times to give an acidity of at least 4%. All transfers are made within 2 to 5 hours after the maximum acidity is reached.

R. Whitaker

BUTTER

416. Method of enhancing the yield of vitamins in fermentation. H. L. POLLARD, N. E. RODGERS, AND R. E. MEADE. (Assigned to Western Condensing Co.) U. S. Patent 2,449,140. 4 claims. Sept. 14, 1948. Official Gaz. U. S. Pat. Office, 614, 2: 433. 1948.

When whey and skim milk are fermented with *Clostridium acetobutylicum*, the vitamin content, particularly riboflavin, is enhanced by the addition of iron and a soluble ammonium salt in a predetermined amount and ratio.

R. Whitaker

417. Fermentation method for preparing a vitamin concentrate. H. L. POLLARD, N. E. RODGERS, AND R. E. MEADE. (Assigned to Western Condensing Co.) U. S. Patent 2,449,143. 3 claims. Sept. 14, 1948. Official Gaz. U. S. Pat. Office, 614, 2: 434. 1948.

Essentially the same as abstract no. 415, except iron and a soluble manganese salt are employed.

R. Whitaker

418. Method of improving the yield of riboflavin in fermentation processes. H. L. POLLARD, N. E. RODGERS, AND R. E. MEADE. (Assigned to Western Condensing Co.) U. S. Patent 2,449,142. 4 claims. Sept. 14, 1948. Official Gaz. U. S. Pat. Office, 614, 2: 434. 1948.

Essentially the same as abstract no. 415, except iron and zinc are employed.

R. Whitaker

419. Production of riboflavin by fermentation processes. H. L. POLLARD, N. E. RODGERS, AND R. E. MEADE. (Assigned to Western Condensing Co.) U. S. Patent 2,449,141. 2 claims. Sept. 14, 1948. Official Gaz. U. S. Pat. Office, 614, 2: 433. 1948.

Essentially the same as abstract no. 415, except iron and a soluble magnesium salt are employed.

R. Whitaker

BUTTER

420. Die Beseitigung des Öl-und Fischgeschmackes der Butter durch pH Regelung. (The removal of oiliness and fishiness in butter by pH regulation.) (English summary.) NIS PETERSEN. Die Milchwissenschaft, 3, 1: 12-17. 1948.

The authors were unable to correlate the reductase test of milk with oiliness in butter made from the tested milk. Pasture feeding tended to increase incidence of oiliness in butter as compared with feeding hay. Preventive measures against oiliness and fishiness in butter are: (a) mixed feeding ration, (b) high pasteurization temperature, (c) limited washing of butter, (d) low salting of butter, (e) short, rapid working of butter, (f) pH 6-7, (g) protection from light, (h) protection from metal, (i) use of anti-oxidants (wheat germ oil, nordihydroguaiaretic acid, etc.).

I. Peters

421. Das Ausölen der Butter. (The oiling-off of butter.) (English summary.) W. MOHR AND K. BAUR. Die Milchwissenschaft, 3, 1: 17-23. 1948.

The oiling-off of butter was measured at 25 and 28° C. Six cubes of butter (1 cubic inch each) were placed on weighed unhardened filter paper (area 5 to 10 times that of the butter cube) and held at 25 to 28° C. for 48 hours, cooled at 10° C. for 30 minutes and the filter paper re-weighed. Butter held at 28° C. was considered to show low oiling-off if 15% or less fat was absorbed by the paper in 48 hours, whereas a 30% loss or more shows high oiling-off. Factors found to decrease oiling-off were: (a) low melting point of the fat, (b) low air content in butter, (c) use of alfa buttermaker as compared with Fritz or churn buttermaker.

I. Peters

422. Das physikalische Bild der Butter. (The physical aspect of butter.) (English summary.) N. KING AND W. FRITZ. Die Milchwissenschaft, 3, 1: 2-12; 3, 2: 36-41. 1948.

The authors studied the physical structure of butter microscopically. A known quantity of butter to be examined was stirred into a given volume of butter oil containing 1 to 1.2% octyl alcohol and placed in a counting chamber 0.01 mm. deep, covered with a cover glass and examined under polarized light filtered through a red filter. The fat globules showed a double ring border. Magnification of 400 was used and the temperature of the butter maintained rigidly between 14 to 16° C.

Alfa butter showed the highest per cent of uniform, small, round fat globules with crystals at the border only, whereas machine butter showed larger, slightly flat or irregularly shaped fat globules with crystal formations within the globules. The churn butter took an intermediate position between the alfa and machine butter. The alfa butter had the lowest per cent of free fat and air, followed by the churn and machine butters, respectively.

Butter cubes held at 30° C. for 9 hours and refrigerated over night showed marked difference to cutting resistance. The alfa and machine butter when cut showed a smooth-cut surface, whereas the churn butter was crumbly and difficult to cut. Both the machine and churn butter showed greater melting resistance than did the alfa butter when held at 30° C.

I. Peters

423. Production methods and the keeping quality of churning cream. . . H. R. THORNTON, R. K. SHAW, AND F. W. WOOD. Univ. of Alberta, Edmonton, Can. Sci. Agr., 28, 9: 377-392. Sept., 1948.

Samples of farm-separated churning cream were collected at 1 creamery and from 2 farms, one producing a high quality cream from machine-drawn milk, while the other ordinarily produced a much lower quality cream. Two additional samples of cream also were secured at the latter farm after the utensils and separator parts had been sterilized. Sub-samples from these were held at temperatures ranging from 40-60° F. for periods up to a month. These

were tested at intervals for off-flavour development, titratable acidity, methylene blue reduction time and bacterial plate count.

Unsterile surfaces with which the cream came in contact, together with storage temperatures above 50° F., were responsible for cream samples dropping from the special grade flavour category. Storage at 50° F. will maintain cream in special grade if delivered twice weekly, while 45° F. storage will maintain this grade for 1 week. The titratable acidity test is of limited value as a means of grading such creams, while the reduction test and bacterial plate count are of no advantage in the routine control of churning creams. Cream may be special grade for churning purposes and yet contain maximum numbers of bacteria.

O. R. Irvine

CHEMISTRY

424. Fosfataseenzymets varmedestruktion. (Destruction of the phosphatase enzyme by heat.) (English summary.) K. P. ANDERSEN, A. M. MADSEN, G. WITTIG, AND H. FAXHOLM. 57. Beretning fra Statens Forsøgsmejeri., Hillerød, Denmark. 1948.

A method for the determination of the laws which govern the effect of heat and time on the phosphatase enzyme was worked out.

A small glass ampule was used for the pasteurization of 1 ml. of milk. The ampule was 100 mm. high, of which 50 mm. was neck. The outside diameter of the neck was 5.0 mm., of the lower part 19 to 20 mm. The thickness of the glass wall was 0.5 mm. The heat conductivity of the glass ampule was 0.026. Heating of 1 ml. of milk 20° C. \pm 0.1° C. would take a calculated 17.1 sec., making it impossible to use direct heat at temperatures so high that the time of destruction would be shorter than time of heating. The cooling of 1 ml. milk from 80 to 60° C. was calculated to take 0.94 sec. in ice water. The destruction of the phosphatase enzyme in this time and at the corresponding temperatures is very insignificant.

These calculations showed it impossible to state the pasteurization time at high temperatures by heat transmission. To overcome this, part of the milk, in which the phosphatase enzyme already was destroyed, was heated to such a temperature that addition of the rest of the milk would bring the temperature down to pasteurization temperature. By churning of raw cream, a buttermilk with a phosphatase activity of 5-6 times that of raw milk was obtained, and this was used where dilution was necessary. The difference in destruction of the phosphatase enzyme in the concentrate and in the corresponding milk was not greater than between 2 different samples of raw milk. The enzyme concentrate was preserved with 1% potassium dichromate in order to carry the whole experiment out with the same concentrate. The preservative caused only a slightly faster destruction of the enzyme as compared to normal milk.

The technic for pasteurizing the milk was that 0.75 ml. of pasteurized milk was preheated to a temperature such that injection of 0.25 ml. of enzyme concentrate at 37° C. would bring the mixture down to the desired holding tem-

perature, following which it was transferred quickly to a water bath of desired temperature and when time was out transferred quickly to ice water. The method was used between 60–80° C. All transfers were made as fast as possible (0.3 sec.). Where the time of destruction was shorter than 1 sec., the transfer to a holding bath was left out.

The speed of reaction of the enzyme was determined at constant temperature ranging from 60–80° C. The results obtained showed that destruction of the phosphatase enzyme is not a first order reaction. The phosphatase enzyme has an optimum stability at its natural pH, compared to pH 6.2 and 7.4. The increase in rate of destruction caused by increase in temperature is found to be in agreement with Arrhenius' formula. Calculations showed that for 5° C. increase in temperature the increase of destruction was 10 times as great, which was in good agreement with results found experimentally. From the results obtained, the time of destruction can be calculated for any pasteurization method.

T. Kristoffersen

425. Trace sugars in milk. C. P. ANANTAKRISHNAM AND B. L. HERRINGTON.
Dept. Dairy Industry, Cornell Univ., Ithaca, N. Y. Arch. Biochem.,
18, 2: 327–337. 1948.

By means of fractional crystallization, glucose was isolated from dialyzed, de-ionized milk serum. It constitutes the whole of the monoses in milk, and exists in the free state to the extent of 4.08 to 7.58 mg./100 ml. of milk (10 samples). Colostrum milk contains more glucose than normal milk. Immediately after calving, the colostrum of a Holstein cow contained 15 mg. glucose per 100 ml. colostrum, that of a Jersey 12.5 mg./100 ml. The Holstein reached a normal glucose value in 3 days, the Jersey in 10 days after parturition.

H. J. Peppler

426. Some effects of feeding synthetic thyroprotein to dairy cows. C. E. ALLEN, DOROTHY S. DOW, V. S. LOGAN, AND C. D. MACKENZIE. Cent. Exptl. Farm, Ottawa, Can. Sci. Agr., 28, 8: 340–356. Aug., 1948.

In a 3-month trial, a synthetic thyroprotein, 'Protomone', containing 3.07% thyroxine by analysis was fed to 3 Holstein and 3 Ayrshire cows at the rate of 15 g. daily for 6 weeks and the effects compared to paired controls. The results include data on the 2-week period preceding treatment and the 4-week period after treatment was completed. Increased milk production occurred in the early part of treatment, the maximum individual increase over the pre-treatment period being 16.7%. All cows, with one exception, declined rapidly subsequent to treatment. Fat tests were increased by from 0.4 to 1.4%, but no significant changes occurred in lactose, total nitrogen, ash or solids-not-fat percentages. Milk yields calculated on a 4% fat basis showed marked increases, the maximum being 41.2%. Body weight losses varying from 7.1 to 13.7% were observed in all cows treated despite the addition of 2 lb. of extra meal per cow per day. Pulse rates were markedly higher for the treatment period but declined after treatment to less than the control animals. No other evidence of ill-health was evident, however.

The need for longer term investigations of the effect of thyroprotein on dairy cattle is apparent before use of this substance can be recommended. The necessity for its strict control to prevent dishonest production records also is pointed out.

O. R. Irvine

427. Choline content of live stock feeds used in western Canada. L. W. McELROY, H. A. RIGNEY, AND H. H. DRAPER. Univ. of Alberta, Edmonton, Can. Sci. Agr., 28, 6: 268-271. June, 1948.

The choline content of a number of feeds of different types was found by preparing a solution of extracted choline reineckate in acetone and determining the amount present in an Evelyn photo-electric colorimeter, using filter no. 515. Samples of grain, hay, by-products and commercial poultry feeds were examined and the results recorded. Oats and barley exceeded wheat and corn as sources of choline. Legume hays were found to be better sources than grass hays and the choline content was higher in leafy good coloured samples. Buttermilk powder and whey powder gave values considerably greater than skim milk powder.

O. R. Irvine

FOOD VALUE OF DAIRY PRODUCTS

428. Discussion on unidentified nutrient. C. A. CARY AND A. M. HARTMAN. U. S. Dept. of Agr. Certified Milk, 23, 9: 6-9. Sept., 1948.

The existence of an unidentified nutrient X was demonstrated by the improvement in growth of rats when certain feeds were fed in or with the basal ration. In general, the nutrient X was found in leafy foods, liver, beef and pork muscle, egg yolk, whole milk, skim milk, non-fat solids of milk, cheese, crude casein from milk and all milk products containing the crude proteins of milk. It was not found in cereal grains, cereal grain products, yeast and yeast products. Experiments also showed that X may be synthesized by bacteria in the rat. However, the rat's requirement must be supplied in the diet because the synthesis by bacteria occurred only under extraordinary conditions and, therefore, is undependable.

W. S. Mueller

ICE CREAM

429. Ice cream dipper. M. F. COSTA. (Assigned to T. N. Benedict Mfg. Co.) U. S. Patent 2,448,863. 1 claim. Sept. 7, 1948. Official Gaz. U. S. Pat. Office, 614, 1: 225. 1948.

An ice cream dipper having a semi-spherical bowl and a scraper blade for discharging the ice cream is described.

R. Whitaker

430. Frozen food container. C. R. SYMMES. (Assigned to H. P. Hood and Sons, Inc.) U. S. Patent 2,444,861. 3 claims. July 6, 1948. Official Gaz. U. S. Pat. Office, 612, 1: 248. 1948.

Ice cream and other frozen foods are frozen in a lined cylindrical carton which is so constructed that the product, surrounded by the liner, may be ejected from the container by upward pressure on the bottom. Peeling of the liner is facilitated by tabs on each side of a lengthwise slit in the liner.

R. Whitaker

MILK

431. Suspending agent for beverages. K. W. KARNOOP. (Assigned to Kalva Corp.) U. S. Patent 2,448,599. 8 claims. Sept. 7, 1948. Official Gaz. U. S. Pat. Office, 614, 1: 162. 1948.

A water soluble extract of Iridophycus is suitable as a stabilizer for chocolate flavored beverages, such as chocolate milk. R. Whitaker

MISCELLANEOUS

432. Cream whipper. E. C. BRULL. U. S. Patent 2,444,897. 1 claim. July 6, 1948. Official Gaz. U. S. Pat. Office, 612, 1: 256. 1948.

The ends of 2 rectangular thin flat sheets of bendable metal are fastened together to form an ellipse in side elevation. This dasher is caused to rotate rapidly by means of a vertical shaft through the center. R. Whitaker

433. Milking machine. A. C. BLOEMERS. U. S. Patent 2,445,904. 7 claims. July 27, 1948. Official Gaz. U. S. Pat. Office, 612, 4: 960. 1948.

A milking machine which has individual control of the vacuum on each teat cup is described. R. Whitaker

434. Milk can drying rack. E. P. SWINTOSKY. U. S. Patent 2,449,628. 1 claim. Sept. 21, 1948. Official Gaz. U. S. Pat. Office, 614, 3: 698. 1948.

A simple angle iron rack for holding lids and milk cans in an inverted position to facilitate drying after cleaning is described. R. Whitaker

435. Milk can strainer accommodating attachment. P. P. HANSON. U. S. Patent 2,450,510. 3 claims. Oct. 5, 1948. Official Gaz. U. S. Pat. Office, 615, 1: 147. 1948.

A rubber gasket is described which fits on milk cans for holding a conventional type milk strainer. Channels between V shaped ribs permit escape of air from the can. R. Whitaker

436. Liquid temperaturizing vat. F. J. McCULLOUGH. U. S. Patent 2,446,054. 4 claims. July 27, 1948. Official Gaz. U. S. Pat. Office, 612, 4: 999. 1948.

A cylindrical pasteurizing vat for dairy products which provides a built-in heating or cooling device is described. A pump circulates the heating or cooling medium from a sump tank spirally upward between 2 walls containing heating or cooling coils and surrounding the vat. On reaching the top, the medium is sprayed inward against the wall of the vat where it flows downward to the sump to be recirculated. R. Whitaker

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